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ANALYSIS AND COMPARISON OF CYANIDE DETOXIFICATION METHODS FROM SPENT HEAPS

A

DISSERTATION

Presented to the Faculty
of the University of Alaska Fairbanks
in Partial Fulfillment of the Requirements
for the Degree of

DOCTOR OF PHILOSOPHY

By
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Fairbanks, Alaska
December 1997

ANALYSIS AND COMPARISON OF CYANIDE DETOXIFICATION METHODS FROM SPENT HEAPS

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ABSTRACT

One goal of this research was to assess bioaugmentation using microorganisms previously isolated at the U.S. Bureau of Mine's Salt Lake Research Center (SLRC) and transported to Alaska. A second goal was to test native strains of microorganisms collected from an Alaskan mine site for cyanide degradation. Another goal of this research was to develop conceptual designs for an in situ biological system to detoxify leached ore heaps. The final goal was to compare the in situ biological system with other common in situ and ex situ heap detoxification processes. To compare the costs associated with the different cyanide destruction processes, a cost comparison, sensitivity analysis, and Monte Carlo simulation were conducted.

Microorganisms from both the SLRC and collected from an Alaskan mine site were tested in the laboratory for cyanide degradation. Once degradation was confirmed, a winter survival rate test and an in situ heap detoxification test were performed using the SLRC sample. Collection, isolation, and cyanide degradation testing of agglomerate samples from the Ryan Lode site indicated that several bacterial colonies could tolerate cyanide in the growth medium, but none were found that could degrade cyanide.

Over-winter survival experiments indicated that approximately 5-20% of the bacterial population in the heap agglomerate samples were viable and survived the winter and subsequent wait in a coldroom. In addition, bacteria colonized the agglomerate samples where nutrients were available.

During August 1993, a 250 ton test heap was constructed on a heap that was

undergoing detoxification using the INCO air-SO_2 process. The test heap was inoculated with bacteria in October 1993 and again in September 1994. Unfortunately, the in situ heap detoxification test was terminated at an early stage in May 1995, limiting the sampling period and conclusions that could be drawn about in situ biological detoxification.

The seven cyanide detoxification processes chosen for comparison were in situ biological, in situ peroxide, in situ chlorination, ex situ biological, INCO air-SO_2, ex situ peroxide, and ex situ chlorination. To compare the detoxification costs of the different processes, a hypothetical spent heap of 1 million tons of ore was used for all calculations. For the scenario analyzed, in situ biological detoxification was two to three times more cost effective than the other methods, with a cost of $0.41 per ton of ore. The method that ranked second was ex situ biological detoxification, with a cost of $0.92 per ton of ore. The remaining methods ranged from $1.05 to $1.35 per ton of ore. The sensitivity analysis indicated that biological detoxification was most sensitive to the rinsing rate, labor costs, and capital costs. The chemical detoxification methods were most sensitive to the oxidant cost, oxidant to cyanide ratio, and starting cyanide concentration. For the Monte Carlo simulation, in situ biological detoxification was the most cost effective, with a detoxification cost of $0.63 per ton of ore. In situ peroxide ranked second with a cost of $1.09 per ton. The remaining methods ranged from $1.11 to $1.45 per ton.

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CHAPTER ONE

1.0 INTRODUCTION

For countless generations, man has been drawn to the element gold. Gold's luster, feel, weight, and malleability have driven people to search and dig into the earth to possess it. J.C. Yannopoulos (1991) writes in his preface:

> The history of gold begins in antiquity. Bits of gold were found in Spanish caves that were used by the Paleolithic people around 40,000 B.C. Gold is the "child of Zeus," wrote the Greek poet Pindar. The Romans called the yellow metal *aurum* ("shining dawn"). Gold is the first element and the first metal mentioned in the Bible, where it appears in more than 400 references.

Our generation is no different. Today, miners have the advantage of using massive equipment capable of moving hundreds of tons of material with each pass. Modern miners say that they are using "state-of-the-art" processes to remove gold from the mountains of material dug out of the earth each year. However, quite the opposite is probably true. Most of the state-of-the-art processes employed at modern mines have probably been around since the turn of the century. What is new is the application of time proven processes to solve old problems in new and novel manners.

One time-proven process which has changed significantly over the last twenty years is the use of cyanide leaching to extract precious metals from ore. One of the most cost effective methods for removing gold from low grade ores is cyanide heap leaching. Throughout the world, the use of cyanide in heap leaching has

revolutionized and revived the mining industry by making it possible to mine mineral deposits which were previously not economical.

In Alaska, several precious metals mines are scheduled to come on-line in the near future, and these mines will probably be using some type of cyanide leaching to remove the gold and other metals from the mined rock.

Like most processes, heap leaching has its drawbacks. One of the most time consuming and highest costs of the leaching process is the destruction or recovery of toxic constituents in the mine wastes. In this dissertation, the new and quickly growing technology of degrading cyanide from the heap leach pads in-place, using bacteria and cost comparisons between competitive chemical methods, will be thoroughly discussed.

1.1 Heap Leaching

1.1.1 History

Cyan, cyanide, and other derivatives stem from the Greek word *kyanos*, which means a bluish-green color. The dye Prussian blue, $Fe_4[Fe(CN)_6]_3xH_2O$, gets its color from the ferrocyanide it contains.

Hydrocyanic acid (HCN), also called hydrogen cyanide or prussic acid, is believed to have been first synthesized by the Swedish chemist Karl Scheele in 1782 (Stanton, Colbert, and Trenholme, 1986). The use of cyanide to leach gold from ore was patented in Scotland in 1880, and was commercialized around 1898, when it was used in vat-type processes at mines in New Zealand and Africa (Smith and Mudder,

1991).

The use of heap leaching to extract precious metals from ore started in the U.S. with research by the U.S. Bureau of Mines in the late 1960's and early 1970's. The research suggested that open heap leaching techniques could be an economical method for extracting precious metals from certain low grade ores, including many waste rock dumps from old mines (Stanton, Colbert, and Trenholme, 1986).

1.1.2 Methodology

The leaching of gold and other precious metals is a process of lixiviation, or washing away the soluble portion. In heap leaching, ore is stacked on top of an impermeable liner and a lixiviant is allowed to percolate through the ore. The ore is usually low grade, containing as little as 0.015 ounces of gold per ton of ore. Because large volumes of the ore are mined and leached, this method is cost effective. In the past five years, several heap leach pads have been constructed to contain more than 10 millon tons of ore, and there are plans in progress for heaps as large as 30 million tons. Figure 1.1 depicts a typical solution circuit for heap leaching, and will be described in more detail in a following section of the text.

When large heap leaching facilities began operating in the mid 1970's, the preferred method for lixiviant delivery was surface sprinkler systems. Because of solution losses to the atmosphere from evaporation and the limited period during the year the solution could be sprinkled, especially in colder climates, the preferred lixiviant delivery system has changed to a stacked drip irrigation system, as depicted

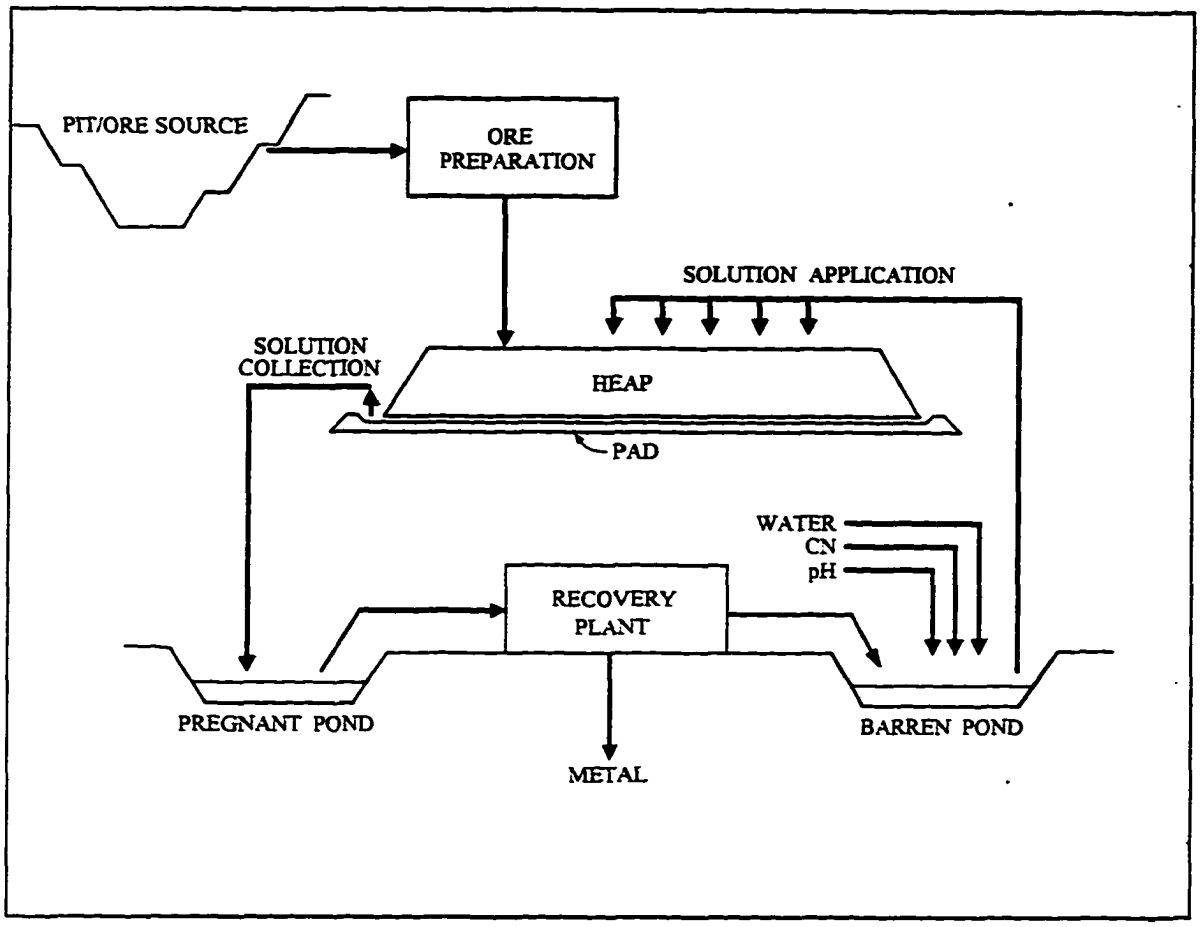


Figure 1.1 Typical solution circuit for heap leaching. (modified from Van Zyl, et al., 1988)

by Figure 1.2. In this system, a series of parallel pipes with emitters every two to five feet is installed in the heap between the successive layers of ore, also referred to as lifts. Figure 1.3 shows the construction of a typical pressure drip emitter. The thickness of the ore lifts is usually five to ten feet, depending primarily on the permeability of the stacked ore. The stacked drip irrigation system can be constructed so that at the onset of cold weather, the lixiviant flow to the upper emitter systems can be turned off, and the lower emitter systems can be used throughout the winter. This

results in production of gold throughout the year, which, although it is reduced in the winter, is still better than a production rate of zero due to the inability of surface sprinkler systems to deliver lixiviant to the heap during the cold months. The stacked drip irrigation system is particularly advantageous in Alaska due to the length and extreme cold of the winters.

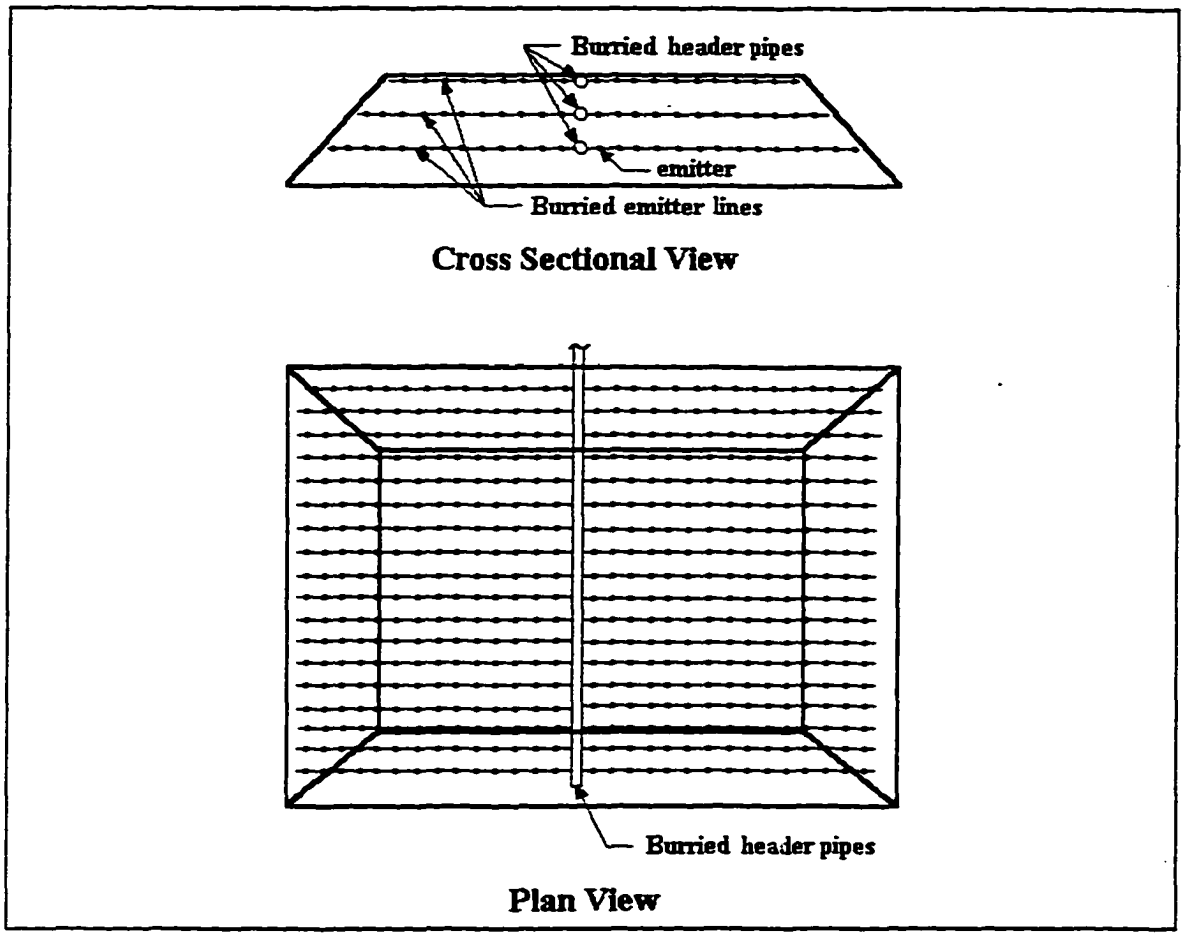


Figure 1.2 Idealized cross section showing a stacked irrigation system.

The lixiviant, usually a solution containing about 500 mg/l of cyanide, is

pumped into the ore through the emitters in the stacked drip irrigation system at the rate of about 0.005 gallons per minute per square foot of heap surface (7.2 gallons per day per square foot). Most mines that heap leach run-of-mine ore add solid sodium cyanide to the ore as it is being stacked on the liner. The sodium cyanide addition rate is dependent on the ore, but is usually one to two pounds of sodium cyanide per ton of ore. Water is then pumped into the heap through the emitters, dissolving the cyanide. Mines which have to agglomerate the ore before stacking the ore on the pad typically add the cyanide to the water that is sprayed into the agglomerator to form the pellets of ore. This usually reduces the time required for the cyanide to diffuse into the ore.

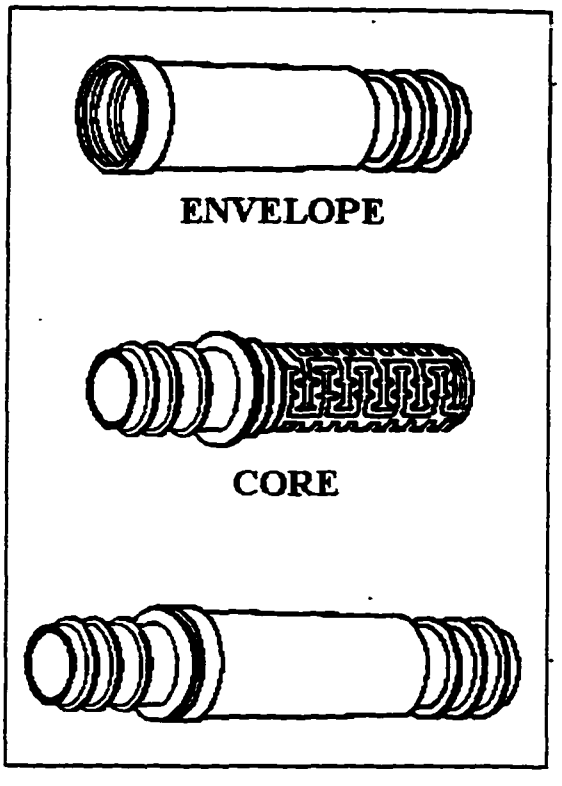


Figure 1.3 Construction of a pressure drip emitter (from Bartlett, 1992).

Once in solution, the cyanide diffuses into the rock matrix of the ore. Diffusion is the physical process which occurs when the concentration of a specific compound in solution is greater than the concentration of the compound in the solids with which the solution is in contact. This difference in the chemical concentration sets the compound in motion as it tries to obtain an equilibrium concentration in the

solids and solution.

Typically, mined ore has a wide range of particle sizes from large blocks to clay-sized particles. In order to determine the time required to leach the ore, the continuum of particle sizes is typically divided into discrete size ranges of particles based on sieve sizes. A model which is used to predict the fraction of gold removed from circular spheres of ore within a discrete size range at various times was solved by Crank (1956)

$$F_{t,r_0} = 1 - \frac{6}{\pi^2} \sum_{n=1}^{\infty} \frac{1}{n^2} \exp\left(- \frac{D_{eff}\, n^2\, \pi^2\, t}{r_0^2} \right) \qquad (1.1)$$

where F_{t,r_0} is the fractional extraction, n is an index which is incremented from one to infinity, r_0 is the radius, and t is time. The effective diffusivity (D_{eff}) is calculated using the following equation

$$D_{eff} \equiv \frac{D\,\epsilon}{\tau} \qquad (1.2)$$

where D is the diffusivity in cm^2/day, ε is the fractional porosity (internal fractional volume of water filled open pores) and τ is the tortuosity of the pores (Bartlett, 1992).

After the cyanide enters the rock matrix, it dissolves the gold and forms gold-cyanide complexes and complexes with other metals. Yannopoulos reviews the theories that have been proposed to explain the mechanism for dissolution of gold in a sodium cyanide solution. L. Elsner recognized in 1846 that oxygen was required for gold dissolution. He proposed the following equation, also known as the oxygen

theory or Elsner's equation:

$$4Au + 8NaCN + O_2 + 2H_2O = 4NaAu(CN)_2 + 4NaOH \quad (1.3)$$

In papers published in 1888 and 1892, L. Janin proposed that hydrogen gas was formed during the dissolution of gold. This became known as the hydrogen theory, described by the following reaction:

$$2Au + 4NaCN + 2H_2O = 2NaAu(CN)_2 + 2NaOH + H_2 \quad (1.4)$$

G. Bodlander published his hydrogen peroxide theory in 1896. He suggested that gold dissolution occurs in a two-step process according to the following equations:

$$2Au + 4NaCN + O_2 + H_2O = 2NaAu(CN)_2 + 2NaOH + H_2O_2 \quad (1.5)$$

$$H_2O_2 + 2Au + 4NaCN = 2NaAu(CN)_2 + 2NaOH \quad (1.6)$$

$$4Au + 8NaCN + O_2 + H_2O = 4NaAu(CN)_2 + 4NaOH \quad (1.7)$$

It can be seen that the first two equations (1.5 and 1.6) add up to Elsner's equation (1.3). Generally, it is believed that Bodlander's first equation (1.5) is the major mechanism for gold dissolution, and that Elsner's equation (1.3) is also important, but a minor mechanism for gold dissolution.

The dissolution rate of gold depends on many factors, including the cyanide concentration, the pH of the solution, and the temperature. The optimum cyanide concentration is usually near 0.05% (500 mg/l) and the optimum pH is near 10.3 (Huiatt, 1984).

After the lixiviant has percolated through the heap and complexed with the gold, it is collected from the bottom of the heap through a system of perforated pipes that drain freely into the pregnant pond. So-called because it contains solution with the gold-cyanide and other metal-cyanide complexes, called the pregnant leach liquor. The pregnant leach liquor is then pumped from the pregnant pond to the stripping plant, where gold is removed from the pregnant leach liquor. There are several methods for removing the gold-cyanide complexes from the pregnant leach liquor, including zinc cementation (Merrill-Crowe process) and adsorption onto granular activated charcoal (GAC). The GAC process typically uses a series of stripping columns in an up-flow circuit, where the gold-cyanide complexes are adsorbed onto the GAC. Once the GAC in a column is "loaded", meaning it has adsorbed as much gold as possible, it is removed from the circuit. The columns are usually rearranged so that the fresh column of GAC replaces the one removed, and is the first column in the series. The columns removed from the stripping circuit are washed with a hot caustic solution to remove the gold-cyanide complexes from the GAC. Once in solution, the gold is removed from the complexes by passing the solution through an electrowinning or plating cell, and the gold is plated onto steel wool or other cathodes. When the cathode can no longer remove the gold from solution, it is placed in a crucible with other chemicals and smelted. The molten liquid is poured into molds and allowed to cool into bars. It is a mixture of gold, silver, and other metals, and is known as Doré metal.

After the pregnant leach liquor has passed through the carbon stripping circuit

and electrowinning cell, it contains little or no complexed gold-cyanide, and is referred to as the barren solution or raffinate. Cyanide is added until the concentration has been brought up to about 500 mg/l, and lime or other alkaline substances are added to keep the pH between 10.5 and 11, minimizing the formation of hydrogen cyanide gas (HCN). After mixing, the barren solution can be returned to the heap and pumped through the stacked ore.

After several leach cycles the gold production from the heap becomes so low that it is no longer cost effective to treat the pregnant leach liquor, and heap decommissioning begins.

In heap decommissioning, the first thing that must take place is the removal or destruction of the cyanide in the heap. The most common practice used today in the mining industry is to remove the cyanide from the heap by rinsing with clean water. Rinsing is measured in pore volumes, a pore volume is the volume of the voids in the heap. The rinse water is collected from the heap through the network of slotted drain pipes described above. The water is usually pumped to a pond where the cyanide is destroyed using chemicals. Once the cyanide is destroyed, the rinse water can be pumped back to the heap for additional rinsing, removing more cyanide as it passes through the heap. After mining, rinsing is probably the most time consuming process at a mine which uses heap leaching.

1.2 The Nature of Cyanide

Cyanide contains two essential elements of life: carbon and nitrogen (Smith

and Mudder, 1991). These elements are combined by a highly energetic triple bond. Bacteria and other organisms mainly attack this compound to obtain the nitrogen for nutrients, and can also use the carbon. The end products of the breakdown of cyanide are typically carbon dioxide and nitrogen, usually in the form of ammonia or nitrates.

Cyanide is found in many natural sources in the environment, including peach pits, lima beans, and even vitamin B_{12}. C.J. Knowles and A.W. Bunch (1986) report that:

> Over 2000 species of plants produce cyanogenic glycosides and other cyanogenic compounds (Conn, 1980). The reason for production of these compounds are not known. Injury to the plant cells by water stress, harvesting, disease and infection, causes release of cyanide (Conn, 1981; Vennesland, *et al.*, 1982). A function for the cyanogenic glycosides could be to protect the plant from predation by micro-organisms. If this is the case, then the degree of protection afforded would depend on the relative damage caused by the cyanide released to the plant tissues and to the phytopathogenic micro-organism (Fry and Meyers, 1981).
>
> Successful invasion of the host plant could occur if the invading pathogenic micro-organism was able to stimulate the breakdown of the cyanogenic glycosides of the host plant, and was itself cyanide resistant.

In the environment, cyanide in low to medium concentrations is short-lived, persisting anywhere from a few weeks to a year. Natural processes convert cyanide to its less toxic forms very rapidly. Some of the natural processes which degrade cyanide are listed in Table 1.1 and are described in the following text.

Table 1.1 Processes for cyanide removal (from Huiatt, et al., 1982).

Natural Degradation	Dilution	
	Volatilization	
	Biodegradation	
	Oxidation	
	Precipitation of Metals	
Oxidation Processes	Alkaline Chlorination	Chlorine Gas
		Hypochlorites
		Electrolytic Generation
	Ozonation	
	Hydrogen Peroxide Oxidation	
	INCO Air-SO_2	
Acidification/Volatilization/Reneutralization		
Adsorption	Activated Carbon	
	Ion Exchange	
	Ion Flotation	
	Precipitate Flotation	
Electrolytic Processes	Cyanide Regeneration	
	Cyanide Destruction	
Conversion to Less Toxic Forms	Thiocyanate	
	Ferrocyanate	
Biological Treatment		

1.3 Cyanide Destruction Processes

There are many different processes which can reduce the amount of cyanide in mine wastewater. Table 1.1 indicates that six major categories of processes are commonly used. The seventh process, biological treatment, is used less frequently but has recently been proven successful at several sites.

1.3.1 Natural Degradation

In Canada, it is common to remove cyanide in effluent from conventional milling circuits by using tailings ponds as wastewater treatment lagoons. Echo Bay Mines' Lupin operation in the Northwest Territories uses two ponds in series to reduce the cyanide levels to acceptable levels. Most of the cyanide degradation occurs in the first pond, and the second pond acts as a polishing pond. In 1986, the cyanide concentration in the mill effluent was greater than 200 mg/l, and the concentration of cyanide in the wastewater released from the second pond averaged 0.29 mg/l (Smith and Mudder, 1991).

Several processes occur in the ponds to reduce the cyanide concentration: dilution, volatilization, biodegradation, oxidation, photodecomposition, and metals precipitation. Sunlight also serves to liberate a fraction of the cyanide contained in the complex ferrocyanide ion. Liberated cyanide, cyanide released from other metal complexes, and any excess cyanide ion are converted to volatile hydrocyanic acid, assisted by the progressive lowering of the pH in the pond (Huiatt, et al., 1982).

Table 1.2 describes some of the advantages and disadvantages of using natural

degradation. This type of process can be used in situ on spent heap leach pads, but the time required for the cyanide concentration to drop to the required level would be very long. Because of the low ground temperatures in the arctic and sub-arctic, the process would probably take longer than in more temperate climates. This author would estimate that natural degradation of the cyanide in a spent heap could take as long 15 years, depending on the configuration of the heap.

Table 1.2 Some advantages and disadvantages of using natural degradation (from Smith and Mudder, 1991).

Advantages
A relatively inexpensive method of treating mill effluent in the event the necessary surface area is available.
Residual weak acid dissociable (WAD) cyanide levels of <0.5 mg/l are achievable.
Iron cyanide complexes are photodecomposed if sufficient daylight is available.
There is no known formation of new toxic by-products.
The process is suitable for batch or continuous operations.
The concentrations of residual metals are also reduced.
The process is suitable as a primary treatment system or as a pre-treatment measure.
Disadvantages
Usually requires large surface area/volume ratios for the ponds. At Echo Bay's Lupin mine, the ponds total 3,000,000 square meters (1.16 square miles).
Requires long periods of time compared to other processes.

1.3.2 Oxidation Processes

Three cyanide oxidation processes are commonly used around the world: alkaline chlorination, hydrogen peroxide, and air-SO_2. Another process, ozonation,

appears to have merits, but no references were found to mines that use this process.

1.3.2.1 Alkaline Chlorination

In this process, chlorine gas can be bubbled through the solution, much the same as in treating drinking water or sanitary wastewater. Another popular method for cyanide destruction is dissolving sodium or calcium hypochlorite [$Ca(OCl)_2$] into the wastewater stream. Both of these processes oxidize free cyanide to its less toxic form, cyanate (OCN^-). Smith and Mudder (1991) provide a description of alkaline chlorination.

> The first stage of cyanide destruction utilizing either hypochlorite or chlorine gas involves oxidation of cyanide to the intermediate cyanogen chloride (CNCl) according to one of the following reactions:
>
> $$NaCN + Cl_2 = CNCl + NaCl \quad (1.8)$$
> $$NaCN + NaOCl + H_2O = CNCl + 2NaOH \quad (1.9)$$
> $$NaCN + Ca(OCl)_2 + H_2O = 2CNCl + Ca(OH)_2 \quad (1.10)$$
>
> At the elevated pH of the oxidation, the intermediate cyanogen chloride is rapidly hydrolysed to cyanate according to the following reaction:
>
> $$CNCl + 2NaOH = NaCNO + NaCl + H_2O \quad (1.11)$$
>
> The entire first stage of the oxidation requires approximately 15 minutes at a pH of 10.5. The second stage of the oxidation involves hydrolysis of the cyanate in the presence of chlorine or hypochlorite to ammonia or carbonate according to the following reaction:
>
> $$2NaCNO + 4H_2O = (NH_4)_2CO_3 + Na_2CO_3 \quad (1.12)$$

The hydrolysis requires approximately 1.0 to 1.5 hours, although reaction periods of several hours have been employed. The oxidation of cyanide to cyanate requires approximately 2.75 parts of chlorine per part of cyanide, although in practice the chlorine consumption is much higher.

Table 1.3 gives a list of the advantages and disadvantages of using alkaline chlorination to treat cyanide in mine wastewater.

Table 1.3 Some advantages and disadvantages of using alkaline chlorination (from Smith and Mudder, 1991).

Advantages
Well established process with considerable operational experience and engineering expertise.
Process equipment and control are reliable and reactions are relatively complete and mechanisms understood.
Heavy metals are precipitated as hydroxides, and can reduced to <1.0 mg/l.
Thiocyanate is oxidized and removed, and cyanate and ammonia can be removed through breakpoint chlorination.
Chlorine is available world-wide in several forms.
Free and weak acid dissociable (WAD) forms of cyanide are oxidized producing low residual effluent concentration (<0.5 mg/l).
Process is adaptable to continuous or batch operation and can be used to treat pulps and clarified metallurgical solutions.
Disadvantages
Reagent consumption and costs can be excessive if high concentrations of cyanide and thiocyanate are present.
Process pH must be controlled carefully to avoid release of cyanogen chloride.
Iron complexed cyanides are not removed under ambient conditions.
The end products of alkaline chlorination, including residual free chlorine and chloramines, must be removed, as these compounds are toxic to aquatic life.
Additional treatment processes may be required to remove ammonia, iron complexed cyanides and metals if very low limits are promulgated.
Cyanide is not recovered, but destroyed.
Cyanide in tailings slurries may not be effectively destroyed and/or reagent consumption may be excessive if the solid phase contains significant concentrations of reduced sulfides or iron.

Chlorination is commonly used to remove cyanide from solutions, raffinates, etc. and is suitable for reducing cyanide levels in spent heap pads in an in situ application and was successfully tested in laboratory columns by L.C. Thompson, et al., (1995b).

1.3.2.2 Ozonation

Huiatt, et al., (1982) describe ozonation as follows:

> In the ozonation process, ozone is generated electrically, either from air or from oxygen. Use of oxygen yields twice the ozone concentration at half the power, and there may be some oxidation contribution from the oxygen itself. Hydrogen cyanide, cyanide ion, the complexes of zinc, cadmium and copper as well as thiocyanate are quickly and easily destroyed. The chemical reactions involved in ozonation are described by Liptak (1974) as follows:
>
> Oxidation of cyanide by ozone
>
> $$NaCN + O_3 = NaCNO + O_2 \quad (fast) \qquad (1.13)$$
> $$2CNO + O_3 + H_2O = N_2 + 2HCO_3 \quad (slow) \qquad (1.14)$$
>
> Oxidation of Thiocyanate by ozone
>
> $$SCN^- + O_3 + H_2O = CN^- + H_2SO_4 \quad (fast) \qquad (1.15)$$
>
> Iron cyanides are more difficult to oxidize with ozone. The combination of ozone with ultra-violet light irradiation is reported to permit complete oxidation of ferrocyanide, but greatly increases cost (Prober et al., 1977; Mauk et al., 1976).

If the ozone rich air could be pumped into the bottom of the heap or bubbled into the heap rinsate, this process could be suited to an in situ type of application.

1.3.2.3 Hydrogen Peroxide

The addition of hydrogen peroxide (H_2O_2) to the wastewater stream is another popular process for treating cyanide in mine wastewater. In industrial applications, this process typically requires ratios of two to five times more H_2O_2 than CN^-. In the presence of copper oxides, cyanide is converted into cyanate (OCN^-) by the following reactions (Smith and Mudder, 1991):

$$CN^- + H_2O_2 + Cu = OCN^- + H_2O + Cu \quad (1.16)$$

$$M(CN)_4^{2-} + 4H_2O_2 + 2OH^- = M(OH)_2 + 4OCN^- + 4H_2O \quad (1.17)$$

$$2Cu^{++} + Fe(CN)_6^{4-} = Cu_2Fe(CN)_6\ (solid) \quad (1.18)$$

$$OCN^- + 2H_2O = CO_3^{2-} + NH_4^+ \quad (1.19)$$

In addition to converting free cyanide, this process also oxidizes the weak to moderately strong cyanide complexes of cadmium, copper, nickel, silver, and zinc (Equation 1.17). An added bonus of this process is that the metals are precipitated as hydroxides.

According to Smith and Mudder (1991) the reaction time varies according to the initial weak acid dissociable (WAD) cyanide concentration, the copper and hydrogen peroxide levels utilized, and the copper to cyanide ratio. The reaction typically requires between 20 minutes and four hours.

Table 1.4 lists some of the advantages and disadvantages of using hydrogen peroxide to treat cyanide in mine wastewater.

Table 1.4 Some of the advantages and disadvantages of using hydrogen peroxide (from Smith and Mudder, 1991).

Advantages
Capital costs are less than or equal to those associated with other chemical treatment processes.
The process is relatively simple in design and operation.
All forms of cyanide including the iron complexed forms can be reduced to environmentally acceptable levels.
Heavy metals are significantly reduced through precipitation.
The process is adaptable for batch and continuous type treatment operations.
The process has been employed in the treatment of pulps, clarified process solutions and heap leach rinse solutions.
Technical support is available from both DuPont and Degussa.
The process has been shown effective in bench, pilot plant, and full-scale operations.
Close pH control is not required.
Automation is not necessary, but available if required.
The process does not produce high quantities of waste sludge and does not introduce significant quantities of total dissolved solids.
There are no license fees required.
Disadvantages
Reagent costs and consumption of copper sulfate and hydrogen peroxide can be excessive.
The process does not remove ammonia and thiocyanate.
Additional treatment may be required if residual effluent concentrations of ammonia, thiocyanate, and metals exceed environmentally acceptable levels.
Cyanide is not recovered, but destroyed.

This process has been used for reducing the cyanide levels in spent leach pads by in situ application and was successfully tested in laboratory columns by L.C. Thompson, et al., (1995b). This process will be further discussed in later sections.

1.3.2.4 INCO Air-SO_2

This process, patented by INCO, Ltd., uses sulfur dioxide (SO_2) and requires the input of oxygen into the wastewater to oxidize the cyanide to cyanate by the following reaction:

$$CN^- + SO_2 + O_2 + Cu + H_2O = CNO^- + Cu + H_2SO_4 \qquad (1.20)$$

In this reaction, copper acts as the catalyst and is required for the reaction to take place. The amount of copper required for this reaction is different for every wastewater stream, and depends a great deal on the concentrations of other constituents present. If the required amount of copper is not present, copper sulfate ($CuSO_4$) is usually added to the wastewater stream. The pH for the reaction is controlled with lime or sodium hydroxide (NaOH) and maintained in the range of 8.0-10.0.

> The theoretical reagent consumption for sulfur dioxide and lime are 2.5 mg SO_2/mg WAD cyanide and 2.2 mg CaO/mg WAD cyanide, respectively. In practice, the reagent consumption is usually higher due to several factors. The reaction time varies from 20-120 minutes. The concentration of copper required for continuous treatment is dependent upon the specific chemical characteristics of the wastewater and must be derived empirically through laboratory and/or pilot plant testing. Many of the mine wastewaters in Canada contain copper concentrations significantly higher than 50 mg/l and therefore copper requirements are reduced or eliminated (Smith and Mudder, 1991).

The SO_2 can be added to the wastewater stream in a variety of forms: sulfur

dioxide gas, sodium sulfite, sodium metabisulfite, and ammonium bisulfite. Elemental sulfur can be burned and the off-gas bubbled through the wastewater to complete the reaction. In addition to the cyanide conversion, 10%-20% of the thiocyanate (SCN^-) is converted to cyanate.

Table 1.5 lists some of the advantages and disadvantages of using the INCO Air-SO_2 process to treat cyanide in mine wastewater.

Table 1.5 Some of the advantages and disadvantages of using the INCO Air-SO_2 process (from Smith and Mudder, 1991).

Advantages
The process has been proven in bench, pilot, and full-scale applications and has been proven effective in the treatment of cyanide and metal containing wastewaters.
The process is effective in treatment of pulps as well as clarified barren and decant solutions and heap leach rinse solutions.
The process is suitable for batch or continuous treatment.
Technical support is available from INCO personnel.
All forms of cyanide are removed including the stable iron complexed cyanides.
Capital costs are comparable with other chemical treatment processes.
Heavy metals are removed through precipitation.
Disadvantages
The reagent costs and consumption for the process (SO_2, lime, and copper sulfate) can be high.
Cyanide is not recovered.
Undesirable quantities of calcium sulfate or total dissolved solids can be produced.
A royalty payment is required for the patented process.
Additional treatment may be necessary for removal of total cyanide, thiocyanate, cyanate metals, and ammonia if stringent effluent requirements and water quality criteria must be met.
Strict control of process variables are required, adding to the costs for monitoring and supervision.
Detailed toxicological data is not available for the effluent of the process.

This process could possibly be adapted for reducing the cyanide levels in spent leach pads in an in situ application, but would require a constant oxygen supply into the heap through ventilation or injection of oxygenated water.

1.3.3 Acidification/Volatilization/Reneutralization (AVR)

The recovery and reuse of cyanide from mining solutions through acidification, air stripping, and readsorption was originally known as the Mills-Crowe process, named after its inventors (Lawr, 1929; Dorr and Bosqui, 1950). The process involves acidifying clarified barren solution to convert the cyanide to HCN, then stripping the HCN from solution using air and reabsorbing it from the air stream with a caustic or lime spray. This method results in good recovery of cyanide from the wastewater stream, and has been used at several mines throughout the world. The Flin Flon Mine, operated by Hudson Bay Smelting and Mining Company, operated an AVR plant from 1931 to 1978. In this application, a reduction in total cyanide of 92% was achieved by lowering the cyanide concentration from 560 mg/l to 44 mg/l (Davis, et al., 1946).

Smith and Mudder (1991) list the following simplified chemical reactions for the AVR process:

$$Ca(CN)_2 + H_2SO_4 = 2HCN + CaSO_4 \quad (1.21)$$

$$M(CN)_2 + H_2SO_4 = 2HCN + M^{2+}SO_4^{2-} \quad (1.22)$$

$$HCN|H_2O = HCN|Air \quad (1.23)$$

$$HCN + NaOH = NaCN + H_2O \quad (1.24)$$

$$M^{2+} + 2OH^- = M(OH)_2 \quad (1.25)$$

$$Na_4Fe(CN)_6 + 2CuSO_4 = Cu_2Fe(CN)_6 + 2Na_2SO_4 \quad (1.26)$$

Table 1.6 provides a list of the advantages and disadvantages of using the acidification/volatilization/reneutralization process.

Table 1.6 Advantages and disadvantages of using the AVR process (from Smith and Mudder, 1991).

Advantages
The WAD cyanide concentration of the barren or tailings impoundment water can be lowered to <10.0 mg/L
Cyanide is recovered for re-use and absorption of cyanide does not present technical problems.
Heavy metals are removed through precipitation.
The reagents required, air, lime and sulfuric acid, are easily obtainable.
Process performance is not sensitive to fluctuations in wastewater chemistry.
Iron-cyanide complexes can be removed through precipitation.
The process is applicable to both decant waters and tailings slurries.
Disadvantages
HCN vapor is hazardous and good safety practices must be employed.
Relatively high capital outlays are necessary, compared to partial chemical treatment.
Additional treatment may be required if stringent effluent standards exist for metals and cyanide, and a level of treatment is required.

This process does not appear to be suited for reducing cyanide levels in spent heap pads in an in situ application, but could be used to recover the cyanide from an effluent stream from a spent heap.

1.3.4 Adsorption Processes

There are several processes which can adsorb cyanide and its complexes, such as: activated carbon, ion exchange, ion flotation, and precipitate flotation. After an

extensive literature search, no mines were discovered which currently use any of these processes, but some could possibly have a successful application at some mines.

1.3.4.1 Activated Carbon Adsorption

The use of granular activated carbon to strip cyanide and metal-cyanide complexes has also been explored. It was found that the cyanide was oxidized to cyanate, but that copper or nickel addition was necessary. Because of the high cost of the activated carbon, this process does not appear to be economically viable.

A study by R.G. Kunz, et al., (1978), used powdered, activated carbon in a once-through system to remove cyanide from petroleum refinery wastes. This created the problem of increased copper concentrations in the waste due to the addition of copper to activate the process.

1.3.4.2 Ion Exchange

This process is mainly suited for removal of metal-cyanide complexes from mine wastewaters. Normally, the anion exchange resin discriminates against the uncomplexed, monovalent cyanide in favor of the di- and tri-valent metallocyanide complexes. This results in the uncomplexed cyanide (free cyanide) not being retained in the resin column (Ingles and Scott, 1987).

Because this method does not remove the cyanide ion, it does not appear to be applicable to treating mine wastewater, because a portion of the total cyanide is usually in ionic form.

1.3.4.3 Ion Flotation

Ingles and Scott (1987) describe the ion flotation process as follows:

> Ion flotation resembles conventional froth flotation in that it employs a collector and similar equipment, and the substance to be separated is carried out of the aqueous medium as a froth of air bubbles (Reed, *et al.*, 1971; Nagahama, 1974). It differs in that the substance to be separated is not usually present initially as a solid. The collectors are ionizable surface-active organic compounds, cationic for the flotation of anions, anionic for floating cations. Since cyanide and its metal complexes are anions, cationic collectors are required. These are usually organic amines similar to that of solvent extraction. Since metal cyanide complexes are removed more effectively than cyanide ions, and some of these complexes more effectively than others, it may be desirable to add metal ions to the solution to be treated.

This process could be very useful to extract gold-cyanide or other metallocyanide complexes from mine wastewaters, but would not be suited for removing cyanide from a spent heap pad, unless additional metals are added to the leachate from the heap as described above.

1.3.4.4 Precipitate Flotation

Ingles and Scott (1987) describe the difference between precipitate and ion flotation processes as follows:

> Precipitate flotation differs from ion flotation in that a colloidal precipitate is first formed and then floated, but in the case of cyanides, the one technique merges with the other as a result of interactions between the metals and the cyanide complexes. A laboratory investigation (Reed et al., 1971) showed that

ferrocyanide and nickelocyanide could be floated effectively, while cuprocyanide has been successfully removed from a zinc concentrate thickener overflow on an industrial scale (2,200 m^3/day) by a combination of ion, precipitate, and ultra-fine particle flotation at the Kamioka mine in Japan (Nagahama, 1974).

This process, like ion flotation, would be very useful for recovering gold-cyanide or other metallocyanide complexes, but would not be suited for removing cyanide from a spent heap pad.

1.3.5 Electrolytic Processes

Huiatt, et al., (1982) describe electrolytic conversion of cyanide as follows:

> Electrochemical methods for treatment of cyanide can be divided into three categories. The chemical reactions involved are as follows:
>
> Electroreduction
>
> $$M(CN)_n^{-n+2} + 2e^- = M^o + n\ CN^- \quad (1.27)$$
> (*where M is a divalent metal*)
>
> Electrooxidation (Anodic)
>
> $$CN^- = CN^o + e^- \quad (1.28)$$
> $$2CN^o = (CN)_2 \quad (1.29)$$
> $$(CN)_2 + 2OH^- = CN^- + OCN^- + H_2O \quad (1.30)$$
>
> Electrochlorination

Electrochlorination

Anode reaction

$$Cl^- (aq) = Cl^\circ + e^- \quad (1.31)$$

$$2Cl^\circ = Cl_2^\circ (g) \quad (1.32)$$

Cathode reaction

$$H_2O + e^- = H^\circ + OH^- (aq) \quad (1.33)$$

$$2H^\circ = H_2 (g) \quad (1.34)$$

With the electroreduction (cathodic) reaction, complex metal cyanide ions undergo reduction at the cathode to deposit or precipitate the metal, generating a corresponding amount of cyanide ion. Electroreduction permits recycling of the regenerated cyanide in the treated barren solution to cyanidation to the extent that the water balance will accommodate it. The advantages of the reduction of fouling constituents (cyanicides) to the metallurgy of the process suggests that it could be advantageous to treat all the barren solution destined for recycle. This option does not, however, eliminate the necessity to treat that portion of the barren solution which must be discarded from the mill.

Electrooxidation (anodic) reactions have apparently not been established but the sequence shown above may apply (Kuhn 1971). Thiocyanate is also oxidized, reporting in the effluent as cyanate and sulfate.

In electrochlorination, introduction of sodium chloride into the solution to be treated gives rise to active chlorine either at the electrode or in solution. These react with cyanides to form cyanates, and with thiocyanate to from cyanate and sulfate, as in conventional alkaline chlorination. Chloride ion is regenerated and is therefore again available for charge transfer. The electrolysis should be carried out at 40°-50° C to minimize formation of chlorate at the expense of hypochlorite generation. As with conventional alkaline chlorination, pH control is important.

The ability of electrooxidation and electrochlorination to reduce total cyanide concentration in the effluent to the proposed levels has not yet been demonstrated unequivocally. Electrochlorination appears to approach this goal more closely than electrooxidation. It should be pointed out that if the barren

solution already contains chloride, as is often the case, electrochlorination will in fact be the operative mechanism. As far as is known, none of these electrochemical options results in elimination of ferrocyanide.

1.3.6 Conversion to Less Toxic Forms

1.3.6.1 Conversion to Thiocyanate

Cyanide can easily be converted to thiocyanate (SCN^-) in the presence of excess sulfur. Huiatt, et al., (1982) describe the process as follows:

> Research in progress at the U.S. Bureau of Mines has shown that cyanide is converted to thiocyanate by a mixture of peroxide and thiosulfate at pH 7 to 10. Levels are reduced from 5 mg/l to less than 0.02 mg/l in 2 to 4 hours. Either free cyanide or complexes less stable than iron are handled.
>
> Conversion of cyanide to thiocyanate was reviewed and critically evaluated by Dodge and Reams (1949). Cyanide reacts with polysulfides in solution at 80° C or higher to yield thiocyanate. Lime sulfur (70 percent calcium sulfide, five percent calcium thiosulfate, five percent free sulfur), a commonly used, cheap agricultural fungicide, can be used as the source of polysulfide (Wernlund and Gunick, 1940). Dodge and Reams point out that "subsequent contact of the thiocyanate with acid would release hydrogen sulfide, and contact with chlorine or other oxidizing agent (at low pH) would (again) release hydrogen cyanide." This is a somewhat remote possibility in the case of gold mill decants.

1.3.6.2 Conversion to Ferrocyanide

Huiatt, et al., (1982) describe the process of converting cyanide to ferrocyanide as follows:

> Addition of excess ferrous sulfate to solutions of cyanide and the complex

cyanides of zinc and copper which have been adjusted to pH's between 7.5 and 10.5 converts most of the cyanide to ferrocyanide. This is one of the oldest methods of cyanide disposal (Moir and Gray, 1909). It was critically reevaluated by the U.K. Department of Scientific and Industrial Research in 1947 (U.K. DSIR, 1947). The method worked best with samples containing from 10 to 100 milligrams of HCN per liter, and required a large excess of ferrous sulfate (16 moles per mole of cyanide for cuprocyanide solutions). Cyanide reductions were 88 to 96 percent. Reed, et al. (1971) concluded that the use of iron for complex formation and subsequent cyanide removal required too close control of solution pH to be feasible, and even then was only effective on pure cyanide solutions.

Cominco Limited used ferrous sulfide at the Con Mine on the shore of Great Slave Lake. This process has generated little interest from other gold mills because it does not, in itself, produce an effluent of a quality acceptable for discharge to receiving waters (Ingles and Scott, 1987).

In a paper describing cyanide destruction processes and costs, M. Drozd (1992) states that the use of ferrous sulfate can be cost effective when treating water containing free cyanide. He also indicates that treatment with ferrous sulfate does not affect the weak cyanide complexes of copper, cadmium, or nickel and requires a supplemental destruction process. Thompson, et al. (1995) also tested in situ cyanide removal in laboratory columns using ferrous sulfate with limited success.

1.3.7 Biological Treatment

Because biological treatment is widely used throughout the U.S. for the treatment of sanitary wastewater, and because the process requires very few additional

expensive additives, there is a growing application of this technology for treatment of industrial wastewater.

At most sites, there are a number of natively occurring microorganisms can tolerate or degrade cyanide. Many of these though, can only survive when the cyanide is present in relatively low concentrations, generally less than 100 mg/l.

The U.S. Bureau of Mines Salt Lake City Research Center (SLRC) isolated bacterial strains that have an affinity for cyanide and can survive at high levels of cyanide (> 100 mg/l). The SLRC and Homestake Mining Co. have successfully used isolated strains of bacteria in biological treatment of mine wastewater (Altringer, et al., 1992; Lein, et al., 1991; Lein, et al., 1990). Another company, Pintail Systems Inc. (PSI), successfully decommissioned heap leach pads using bacteria (Thompson, et al., 1995a; Thompson, et al., 1995b; Thompson, 1992; Thompson, 1990; Thompson and Gerteis, 1990). Table 1.7 lists some of the advantages and disadvantages of using biological treatment.

Biological treatment has the ability to be used to treat cyanide in spent heap leach pads in situ, as demonstrated in experiments by the U.S. Bureau of Mines, PSI, and others, and is described in further detail in section 2.1.

Table 1.7 Advantages and disadvantages of using biological treatment (from Smith and Mudder, 1991).

Advantages
The process is simple in design and process control is minimal.
Reagent costs for phosphoric acid and soda ash are very low in comparison to other treatment processes.
All forms of cyanide are treatable including a portion of the stable iron complexed cyanides.
Heavy metals are removed through a combination of adsorption and precipitation.
Thiocyanate and ammonia are oxidized and removed.
The effluent from the biological treatment process has been shown to be environmentally acceptable.
Disadvantages
Capital costs are slightly higher than those of other treatment processes.
The biological treatment process, as with other treatment processes, has not been widely applied or accepted in the treatment of mine wastewaters or metallurgical solutions.
Suitable microbial populations may not be found locally, requiring obtainment of commercial or patented bacteria for which a fee may be charged.
Additional treatment may be required if residual effluent metals concentrations exceed environmentally acceptable levels.
The performance of the process is affected more adversely by cold temperature than are other chemical or physical treatment processes.
Cyanide is not recovered.
The process is less suitable for batch treatment applications than the other chemical or physical treatment processes.

CHAPTER TWO

2.0 PREVIOUS TESTING

To adequately complete an economic analysis of two cyanide destruction techniques, some background material must be provided to describe previous testing and it's bearing on this research. Therefore, the following sections will discuss previous testing that has taken place in Alaska and elsewhere.

2.1 Heap Rinsing

Bakshi (1992) conducted three column rinse tests on agglomerate from the Ryan Lode site (described in Section 3.1) to determine the time and rinse volume required to reach the specified weak acid dissociable (WAD) cyanide level for detoxifying the cyanide from spent heap leach pads. The first two tests used small columns, 10.2 cm in diameter and 122 cm long, constructed of a length of glass pipe. The agglomerate samples taken from the top meter of heap F showed little or no residual cyanide, so a 0.05% (500 mg/l) cyanide solution was added to the columns in a simulated leach cycle, which ran for 120 hours in tests one and two, and for 48 hours for test three.

The first rinse test ran for 198 hours, continuously rinsing the agglomerate in the column at a flow rate of 204 ml/min/m^2. The second test was conducted in an intermittent fashion, with the pump alternately running for 22 hours, and then being switched off for 22 hours, for a total length of 242 hours.

The third test employed the use of a large, square, clear acrylic column, 30.5 cm on each side by 244 cm long. This test ran for a total of 214 hours, but rinsing was interrupted several times due to pump failure.

Bakshi determined that the time required to reduce the cyanide levels in a heap using intermittent rinsing would be longer than using continuous rinsing. However, the rinse volume would be about one-third less for intermittent rinsing. This lowered rinse volume could result in substantial savings in the pumping and water treatment costs. The conditions used during the tests are outlined in Table 2.1. The WAD cyanide concentrations for tests one through three are provided in Tables 2.2 through 2.4, and plotted in Figures 2.1 and 2.2.

Table 2.1 Testing conditions (from Bakshi, 1992).

	Test 1	Test 2	Test 3
Column Height	122 cm	122 cm	244 cm
Column Shape	round, 10.2 cm	round, 10.2 cm	square, 30.5 cm
Sample Weight	12.5 kg	12.5 kg	256 kg
Solution Flow Rate	204 ml/min/m^2	204 ml/min/m^2	204 ml/min/m^2
Leaching Time	120 hours	120 hours	48 hours
NaCN Consumed	6.0 g	6.0 g	27.5 g
Rinsing Time	198 hours	242 hours	214 hours
Rinse Volume	19.8 liters	13.2 liters	41.4 liters
Cycle Time	22 hours	22 hours	34 hours
Pore Volume	2.2 liters	2.2 liters	38.6 liters

Table 2.2 Results for column test one (from Bakshi, 1992).

Number of Pore Volumes	Rinse Volume (liters)	Rinse Time (hours)	Effluent pH	Effluent WAD Cyanide Concentration (mg/l)
1	2.2	22	9.42	98
2	4.4	44	9.36	44
3	6.6	66	9.28	8.4
4	8.8	88	9.24	3.8
5	11.0	110	9.02	1.4
6	13.2	132	8.86	1.2
7	15.4	154	8.86	0.8
8	17.6	176	8.74	0.4
9	19.8	198	8.62	0.2

Table 2.3 Results for column test two (from Bakshi, 1992).

Number of Pore Volumes	Rinse Volume (liters)	Rinse Time (hours)	Effluent pH	Effluent WAD Cyanide Concentration (mg/l)
1	2.2	22	9.46	96.5
2	4.4	66	9.34	52.2
3	6.6	110	9.28	5.1
4	8.8	154	9.02	2.7
5	11.0	198	8.90	0.9
6	13.2	242	8.72	0.2

Table 2.4 Results for column test three (from Bakshi, 1992).

Sample Number	Rinse Volume (liters)	Rinse Time (hours)	Effluent pH	Effluent WAD Cyanide Concentration (mg/l)
1	4.54	24.5	9.42	114
2	13.63	48	9.38	110
3	28.77	71	9.26	100
4	52.24	96	8.98	84
5	75.71	119	8.86	15
6	102.96	143	8.72	9
7	128.70	166	8.68	0.9
8	155.95	190	8.52	0.5
9	183.21	214	8.40	0.2

The WAD cyanide concentration versus the number of pore volumes of rinse solution is not a linear function (Figure 2.1). Smith and Mudder (1991) indicate that the cyanide concentration during heap rinsing should follow a first order exponential decay curve, and provide Figure 2.3, a semi-log plot which indicates the relationship between the cyanide concentration and the number of pore volumes of rinse solution. A first order exponential decay curve should have the form of Equation 2.1

$$Y = A * e^{(-B * X)} \qquad (2.1)$$

where A is the starting cyanide concentration, B is a constant, X is the number of pore volumes of rinsate, and Y is the cyanide concentration. When an exponential decay

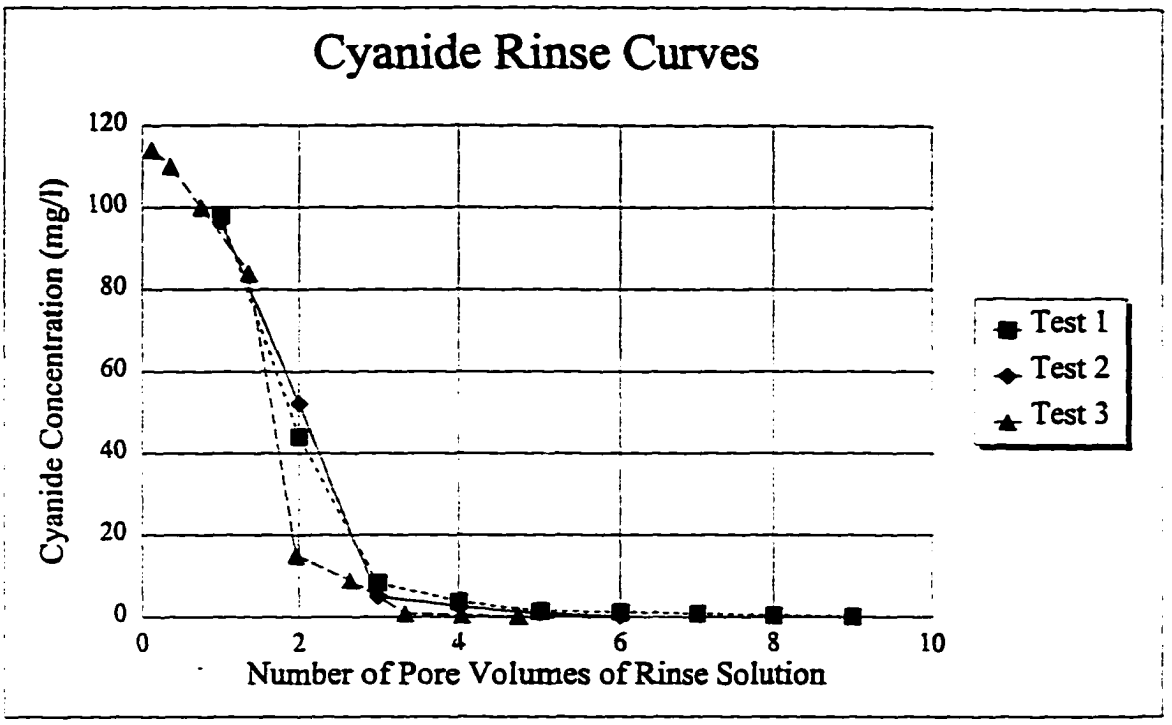


Figure 2.1 Cyanide concentration verses volume of rinsate (from Bakshi, 1992).

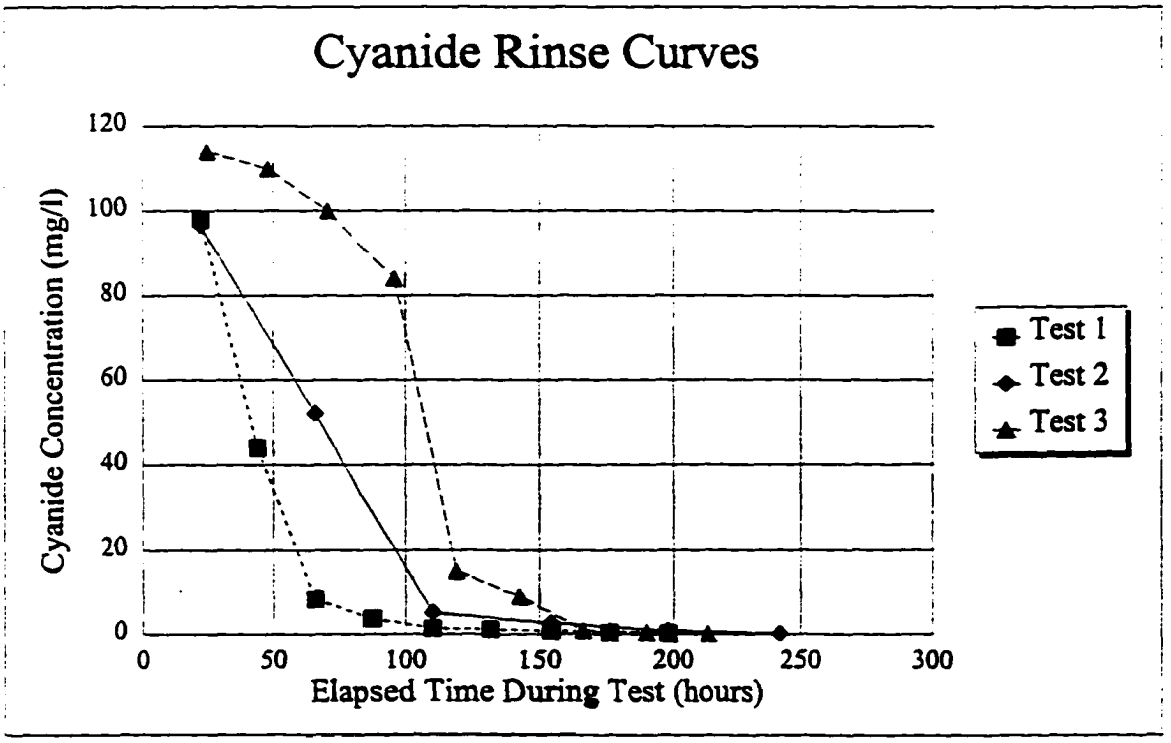


Figure 2.2 Cyanide concentration verses elapsed time (from Bakshi, 1992).

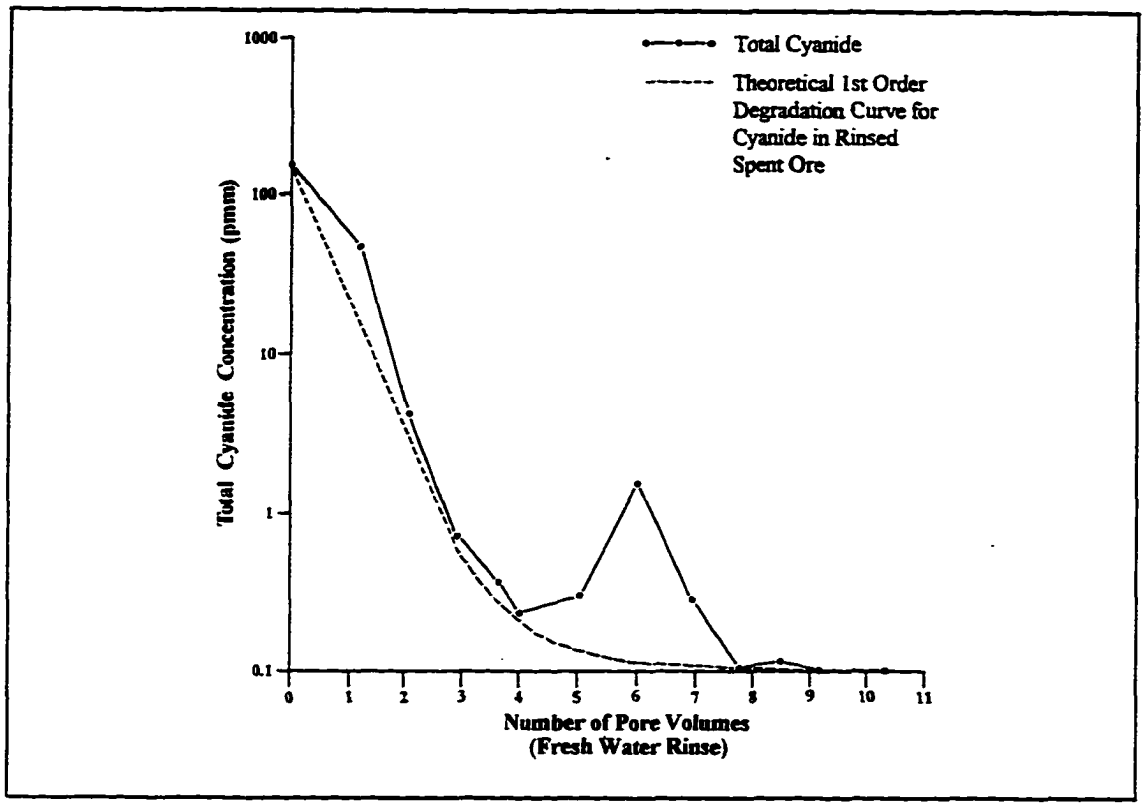


Figure 2.3 Cyanide curve illustrating the delayed release of cyanide (from Smith and Mudder, 1991).

function is plotted in semi-logarithm form as in Figure 2.3, the data should form a straight line. Since the data provided by Smith and Mudder does not fit a straight line, it was obvious that a first order exponential decay curve could not explain the shape of the curve in Figure 2.3 after about three pore volumes. Therefore, the theoretical cyanide concentration over time could not be estimated using a first order exponential decay curve.

After an extensive literature review, a curve was found which was similar to the results from Bakshi's work, and used a third constant, *C*. The equation for the

curve had the general form provided in Equation 2.2

$$Y = A * e^{(-B * X^C)} \qquad (2.2)$$

where A, B, and C are constants, X is the number of pore volumes of rinsate and Y is the cyanide concentration in the heap after X volumes of rinsate.

The NONLIN module from the software program SYSTAT was used to conduct nonlinear regression analysis on the data sets from Bakshi's rinse testing. The data were typed into a file and the NONLIN module was provided the general form of the equation. NONLIN provided the constants for the best-fit line using the general form of Equation 2.2.

When the data from Test three was analyzed, NONLIN reported that the constants were not computable, so it was ignored during the data analysis so that the values of the constants could be computed. The data from all three tests closely matched one another, so the three data sets were combined and analyzed. By combining the data for all three rinse tests into one data set, SYSTAT returned the constants to create Equation 2.3.

$$Y = 109.8 * \exp(-0.111 * X^{2.97}) \qquad (2.3)$$

This equation closely matches the data from Bakshi's work and is plotted in Figure 2.4. Table 2.5 lists the results from SYSTAT for the constants described above for each rinse test Bakshi conducted. This equation will be used in Chapter 4 for predicting the cyanide concentration in a hypothetical heap used for cost estimation.

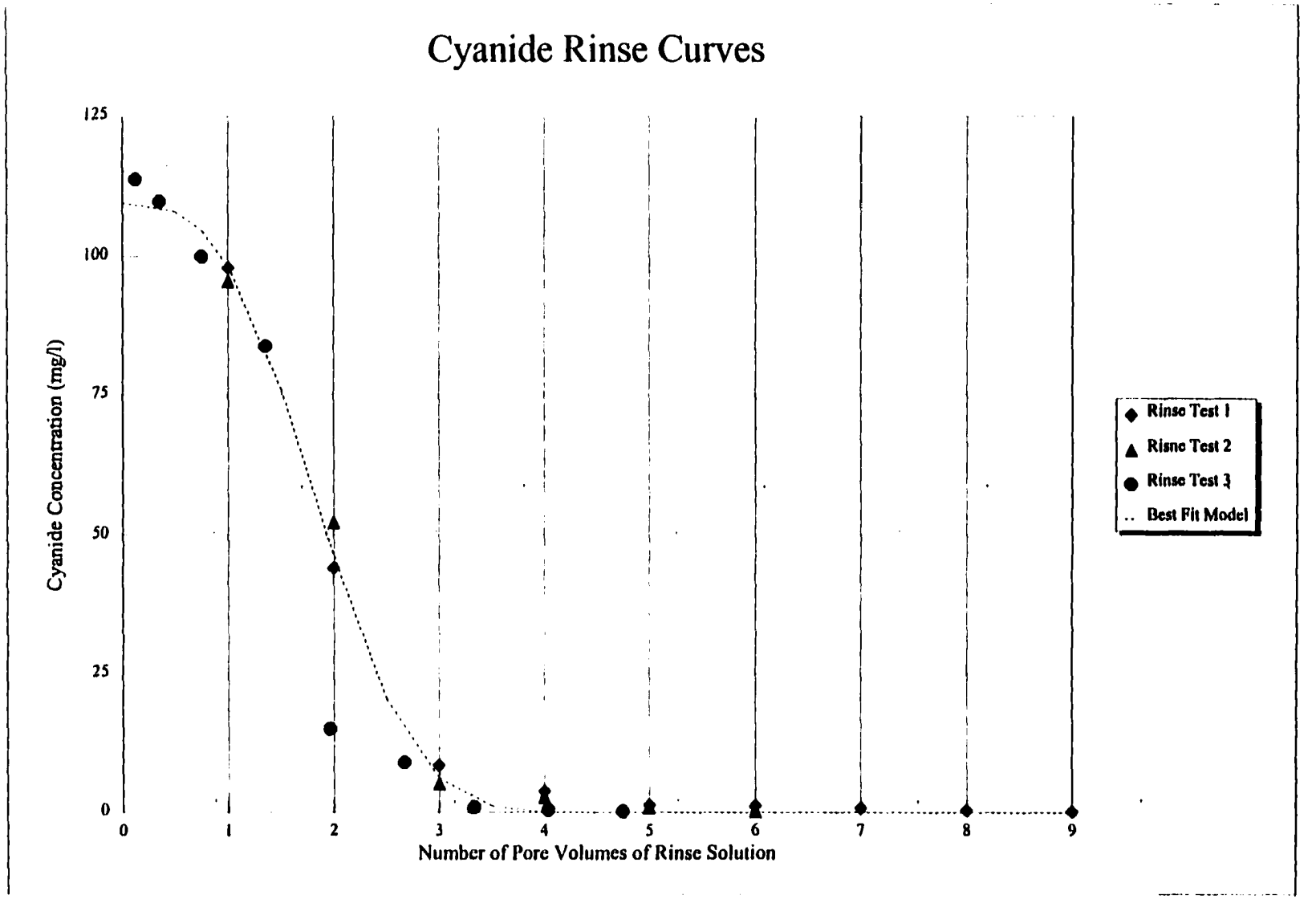


Figure 2.4 Best fit model for cyanide rinsing.

It is likely that the reason the three data sets matched so closely because the tests were conducted on agglomerate gathered from the same heap, and most likely the same area of the heap. Therefore, the material constants for the three tests were nearly identical.

Table 2.5 Nonlinear regression results from Bakshi's experiments.

	A	B	C
Rinse Test 1	121.3	-0.213	2.26
Rinse Test 2	100.4	-0.049	3.73
Rinse Test 3	110.5	-0.119	3.06
Combined	109.8	-0.111	2.97

2.2 Biological Degradation

In the past ten years, there has been significant attention given to applying biological degradation to detoxify cyanide in mine wastes. Several groups of researchers have successfully reduced cyanide levels in both mine wastewater and solid wastes. Homestake Mining Company and the U.S. Bureau of Mines have treated mine wastewater to appropriate clean-up levels (Altringer, et al., 1992; Lein, et al., 1991; Lein, et al., 1990; Whitlock and Mudder, 1986) and Leslie Thompson and her colleagues at Pintail Systems, Incorporated (PSI), have developed a proprietary process which has been successfully applied to test and operational spent heaps (Thompson, et al., 1995a; Thompson, et al., 1995b; Thompson, 1992; Thompson, 1990; Thompson and Gerteis, 1990). These processes will be explained in detail in the following

sections.

2.2.1 Homestake Mining Company

In 1984, Homestake Mining Company placed a biological treatment plant (Figure 2.5) into operation. This plant has probably the best-known and successful application of biological cyanide detoxification of mine wastewater. The process at Homestake utilizes a series of rotating biological contactors (RBCs) to achieve

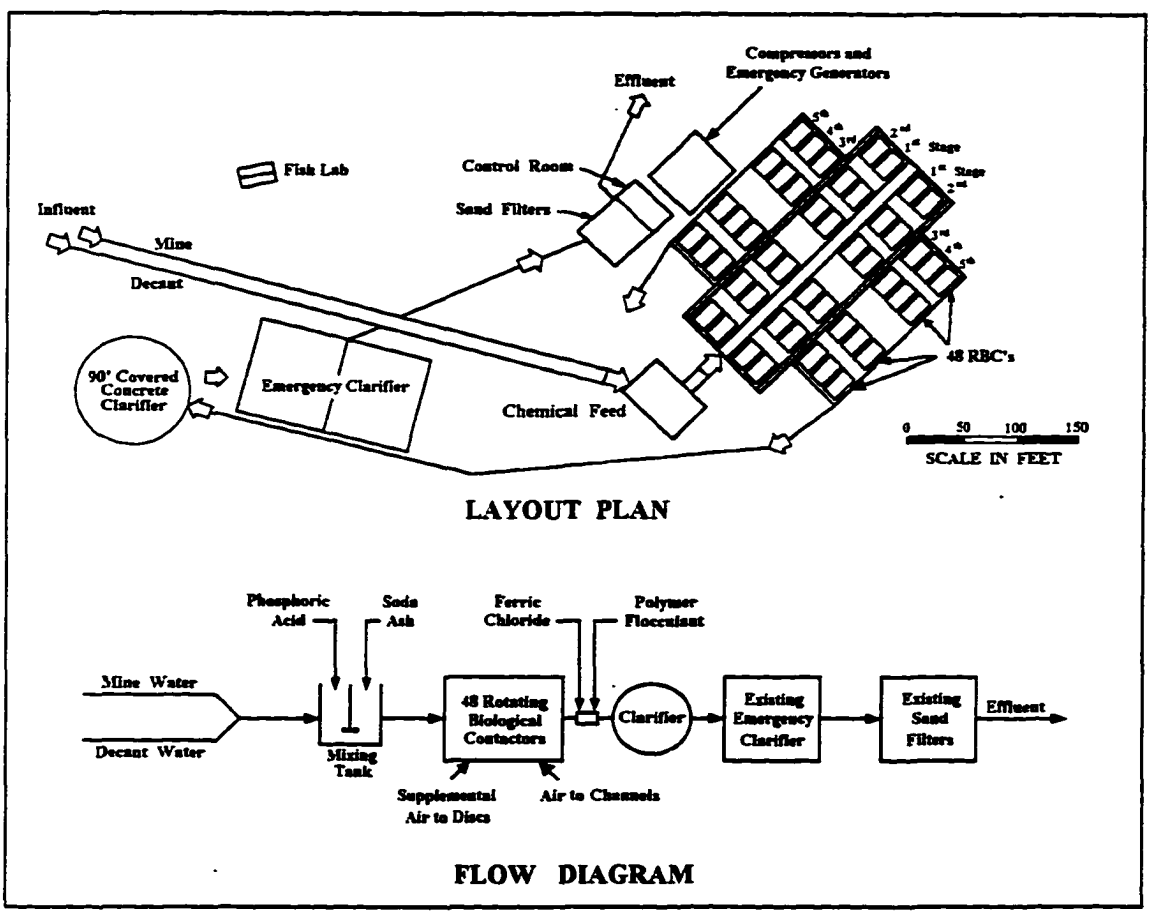


Figure 2.5 Biological treatment works used at the Homestake mine (from Smith and Mudder, 1991).

acceptable cyanide levels. These RBCs are commonly used in sanitary wastewater treatment and are readily available.

The RBCs consist of closely-spaced, thin disks of polyvinyl chloride (PVC) which are typically about half submerged in the wastewater and rotate slowly. The microorganisms attach to the disks and which go through repeated cycles of submersion and exposure. The microorganisms take up nutrients, water, and cyanide while submerged, and then extract oxygen when the rotating disks expose the microorganisms to the atmosphere. This is a continuous flow process where the water travels from one RBC to another, until it meets the required cleanup levels. In addition to treating the cyanide, ammonia, nitrates, and some metals are also removed during treatment in the RBC system.

According to Smith and Mudder (1991), the only nutrients required for this system are an inorganic carbon source (soda ash) to aid nitrification and phosphorous (as H_3PO_4) as a trace element. Since start-up, the quantities of these chemicals added to the process have been consistently reduced and are, at present, used in very limited quantities. As expected, this system had a higher capital cost than chemical treatment systems, but has a substantially lower operating cost and is easier to operate than competitive chemical treatment systems. The operating cost for the Homestake plant averages about $0.25 per 1,000 gallons of wastewater.

Another important factor in using this type of biological system is that it is an ex situ process, which requires the cyanide to be removed from the mine waste prior to oxidation. Therefore, the time required for this system should be identical to ex

situ chemical methods, which rely on heap rinsing.

2.2.2 U.S. Bureau of Mines

In 1992, the USBM's Salt Lake Research Center (SLRC), in conjunction with a privately-owned mine near Ely, Nevada, successfully used biological detoxification for cyanide from mine wastewater. The SLRC conducted three bench-scale laboratory tests and a field test to assess the ability of a sample of microorganisms to degrade cyanide.

In the first bench-scale test, crushed and screened quartz chips were packed into a 2.54 cm diameter, 122 cm long glass column. The wastewater stream, containing 35-45 mg/l of WAD cyanide and low concentrations of peptone, glycerol, and yeast extract (PGY), flowed downward in the column, with a retention time of approximately 4.5 hours. During three of the four tests listed in Table 2.1, the WAD cyanide level in the effluent was reduced to or below 0.1 mg/l. This test also involved the investigation of alternative low cost nutrients, due to the high costs of PGY. It was found that small amounts of phosphate (as phosphoric acid) were effective as a nutrient, with a cost of about 1% of the cost of PGY at the same cyanide oxidation level.

The second bench-scale test used activated carbon with a grain size of 1.75-4.76 mm as the growth surface in a 2.54 cm diameter, 122 cm long glass column. A solution containing 55 mg/l WAD cyanide and 10% PGY was passed up through the column at a rate resulting in a 4.5 hour retention time. The effluent from this test was

also at or below 0.1 mg/l WAD cyanide.

Table 2.6 Results from USBM column test 1 (from Lein, et al., 1991).

Test Number	PGY Concentration (% of Standard)	Effluent WAD Cyanide Concentration (mg/l)
1	10	0.1
2	5	0.1
3	3	0.1
4	2	1.0

The third laboratory bench-scale test was conducted to see if biologically assisted rinsing of spent ore heaps was feasible. This test entailed using two, 10.2 cm diameter glass columns, each 122 cm long filled with 16,500 g of minus 0.64 cm ore. The ore in both columns was leached with process solution from the mine. Column one was rinsed by recycling 500 ml of tap water through the column. Column two was rinsed by passing 500 ml of process water that was passed through the column. Twenty to fifty mg/l H_3PO_4 was added to the effluent from the column, and the solution was treated in an up-flow, 2.54 cm diameter glass column containing 1.27 cm thick bed of activated charcoal to destroy a portion of the cyanide in solution. The retention time was about 13 minutes, and the results, listed in Table 2.7, indicated that biologically assisted rinsing could cut the rinsing time by approximately 25%.

A field test conducted by SLRC used five, 3,028 liter carbon tanks in series, each filled with 909 kg of granular activated charcoal (GAC). This may seem

expensive, but the contrary is probably true. Because activated carbon is used to strip the gold-cyanide complexes from the pregnant solution, there is usually a large amount of GAC on hand at most mines that employ a cyanide leaching process with GAC for gold recovery.

Table 2.7 Results from biological assisted rinsing (from Lein, et al., 1991).

Pore Volume	Bioassisted Rinse Cyanide Concentration (mg/l)	Recycled Rinse Cyanide Concentration (mg/l)
1	5	9
2	1.6	5
3	0.2	0.4
4	< 0.2	< 0.2

Approximately 94.6 liters of inoculum containing the bacterial mix were grown at the SLRC and transported to the mine site. At the site, 19 liters of the inoculum were added to 190 liters of full strength PGY in a 208 liter drum, and allowed to grow for one week in a static state. After growth, 190 liters of inoculum and 225-265 liters of PGY were added to each of the carbon tanks. The tanks were then allowed to grow in a static state for one week. Afterwards, each tank received another 190 liters of PGY and remained static for another week. After the second week, the solution in the tanks was circulated through the tanks at 37.8 liters/min for one day. During the two weeks of growth, phosphoric acid was added to the pond to stimulate indigenous cyanide-degrading bacteria.

After a full day of circulation, a process solution was pumped through the tanks at 190 liters/min. A five mg/l drop in cyanide concentration (from 19 mg/l) was measured in the waste water stream. The flow rate was maintained for two weeks at 190 liters/min. After two weeks, the flow rate was increased to 1,135 liters/min and held there for eight weeks. A drop of 1.5-2.5 mg/l in WAD cyanide concentration was measured. Table 2.8 lists the WAD cyanide concentration in each tank effluent at a flow rate of 1,135 liters/min. The reduction in the effluent WAD cyanide concentration for each tank was not very large, probably due to the short retention time in each tank. Had the flow been separated into several, parallel tank series, the retention time in each tank could have increased, and could have possibly increased the amount of cyanide degraded.

Table 2.8 Results from the SLRC field test (from Lein, et al., 1991).

Sample Location	WAD Cyanide Concentration (mg/l)
Influent	14.3
Tank 1 effluent	14.0
Tank 2 effluent	13.5
Tank 3 effluent	13.0
Tank 4 effluent	12.7
Tank 5 effluent	12.4

This work by the SLRC established some very important points: 1) large

populations of microorganisms can be grown with low growth medium concentrations; 2) for this system, phosphorous (at low concentrations) was the only nutrient limiting microbial growth and cyanide degradation.

2.2.3 L.C. Thompson and Colleagues

Leslie Thompson and her colleagues at Pintail Systems, Incorporated (PSI) in Aurora, Colorado, conducted several column and field trial tests of bacterial degradation, and successfully used this method to detoxify two spent ore heaps larger than one million tons in size. Their success has shown that using bacterial detoxification instead of conventional chemical treatment methods can significantly shorten the time required to detoxify the spent heaps. To accomplish the biological detoxification, Thompson first looks for native cultures of bacteria that can be stimulated or induced in the lab to increase their natural degradation rate. If no native bacteria are found to be compatible with the ore, she checks her collection of about 1,200 different strains to find strains which may be compatible with the mine site. She prefers to inoculate the spent ore heaps with a consortium of microorganisms instead of a single strain. The consortium of bacteria should be able to handle the full range of waste products produced from the cyanide oxidation. Typically, several species of bacteria are needed (cyanide-oxidizers, nitrifiers, and denitrifiers) to successfully reduce the waste products of cyanide oxidation to inert end products.

The first heap PSI successfully detoxified was a 1.3 million ton heap at the Yellow Pine. This mine is operated by Hecla Mining, Inc. just east of McCall, Idaho,

at an elevation of 6,500 feet. The single-use leach pad had lifts of ore stacked to a total height of 114 feet. The biodetoxification of the heap began in March 1992, and was completed by September 1992. The initial WAD cyanide concentration in the effluent solution of 47 mg/l was reduced to 0.2 mg/l at the end of the seven month period (Figure 2.6). The site was challenging due to the low solution temperatures and extreme cold weather conditions throughout the operating season. But the microbial process was still faster than the detoxification times for competitive chemical treatment methods estimated to be two to four operating seasons (Thompson,

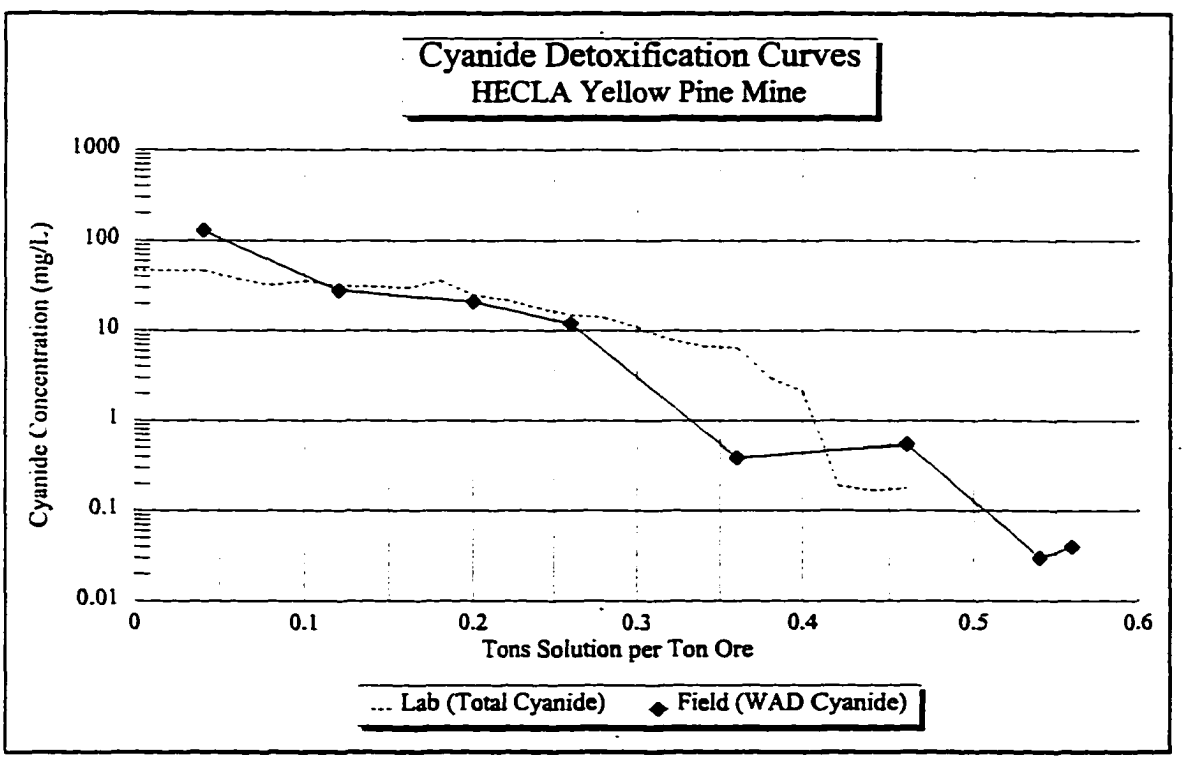


Figure 2.6 Cyanide detoxification curves for the HECLA Yellow Pine Mine (from Thompson, et al., 1994).

et al., 1994).

For the Yellow Pine mine, the detoxification took approximately 0.49 tons solution/ton ore. A comparison of the detoxification curves from the Yellow Pine mine to other published data is difficult because the material properties of the heap were not given in the publication. However, if the properties of the hypothetical heap used for calculations in Chapter 4 are applied to this data, the number of pore volumes of solution needed for detoxification can be calculated using Equation 2.4.

$$V = \frac{\left(\frac{0.49\ tons\ solution}{ton\ ore}\right)(1x10^6\ tons\ ore)\left(2{,}000\ \frac{lb}{ton}\right)}{\left(7.34\ \frac{lb}{gal}\right)\left(37.4x10^6\ \frac{gal}{pore\ volume}\right)} \qquad (2.4)$$

$$V = 3.57\ pore\ volumes$$

This value can be compared to heap rinsing using Equation 2.3 by substituting the starting value of 47 mg/l for 109.8, 0.2 mg/l for Y, and solving for X. In doing so, the required number of pore volumes for heap rinsing was calculated as 3.71 pore volumes. In this case, the difference in the number of pore volumes is not extreme, but the amount of wastewater requiring treatment would be reduced by approximately five million gallons for the hypothetical heap.

The second successful biological heap detoxification by PSI took place at the Cyprus Copperstone Mine, near Parker, Arizona. The heap, a 1.2 million ton single-use leach pad, had an initial WAD cyanide concentration of 30 mg/l. The pad was treated between July and October, 1993 and required a total time of 70 days for

biological detoxification (Figure 2.7). This rapid treatment was probably due to the 80°-90° F temperature of the process solution.

For the Copperstone mine, only about 0.16 tons solution/ton ore were required.

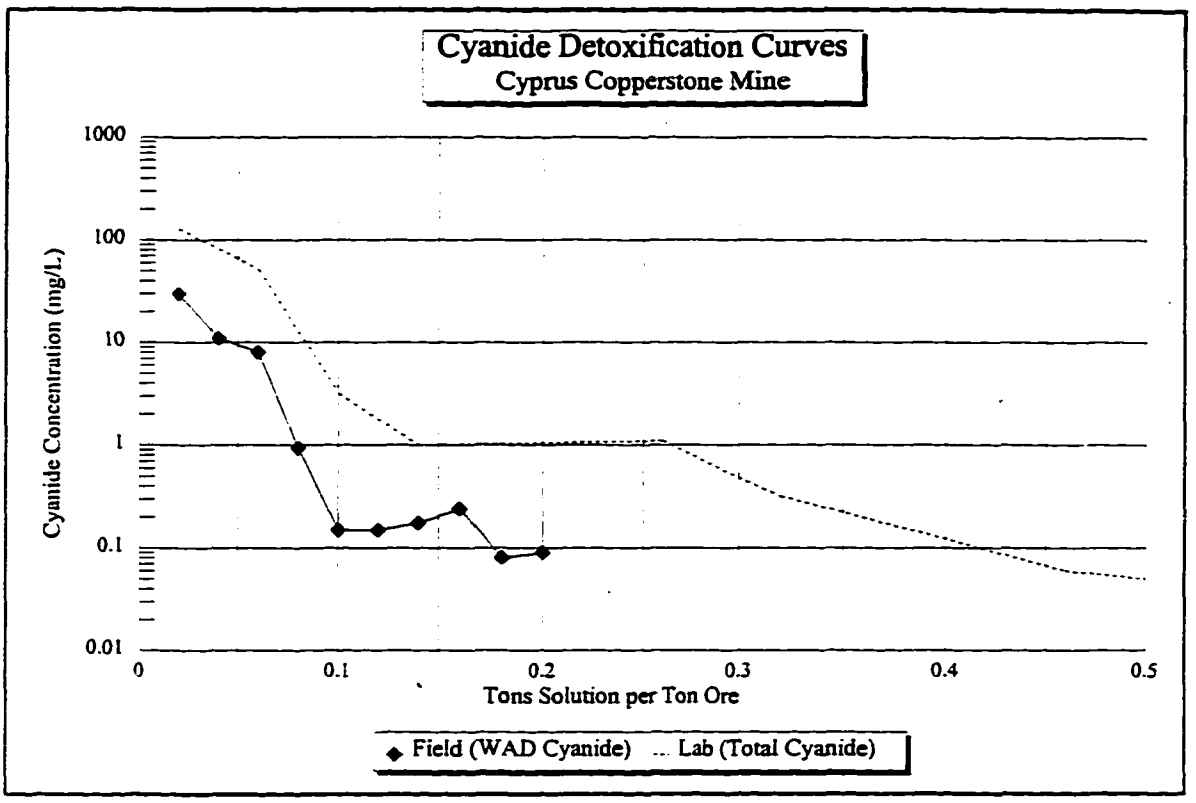


Figure 2.7 Cyanide detoxification curves for the Cyprus Copperstone mine (from Thompson, et al., 1994).

Using the properties of the hypothetical heap and Equation 2.4 the number of pore volumes required for detoxification was calculated to be 1.17. Again using Equation 2.3 and substituting the starting value of 30 mg/l for 109.8 and 0.2 mg/l for Y, the value of X was determined to be 3.61 pore volumes. For the Copperstone mine, the difference in the number of pore volumes for detoxification is more pronounced, and

biological detoxification probably saved Cyprus close to $1 million.

Thompson and PSI also conducted a series of column tests on ore from the Summitville mine in southwestern Colorado. The mine is located 25 miles south of Del Norte, at an approximate altitude of 11,600 feet mean sea level (MSL). Between 1986 and 1992, approximately 10 million tons of ore were placed on a leach pad. Because of unexpected water balance problems and solution discharges, the mining company filed a Chapter VII bankruptcy, and the EPA took over the site in December 1992. The site was added to the National Priorities List in June 1994. PSI and Dames & Moore were awarded a contract to conduct a pilot and field demonstration under the EPA Superfund Innovative Technology Demonstration Program (SITE).

The first series of tests that was conducted, compared biodetoxification of several ore types to freshwater rinsing (Figures 2.8 and 2.9). These curves are important, because they show that the reductions in WAD and total cyanide concentrations in the fresh water rinse column were not as rapid as in the biodetoxification columns.

In the second series of tests, biodetoxification was compared with three competing in situ chemical detoxification methods and freshwater rinsing (Figure 2.10). This important work compared in situ biological to in situ chemical detoxification. In these tests there is a grouping of the data, with biodetoxification clearly being the quickest method, followed by in situ chemical methods ($FeSO_4$, H_2O_2, and $Ca(OCl)_2$) as intermediates, and water rinsing as the slowest process tested.

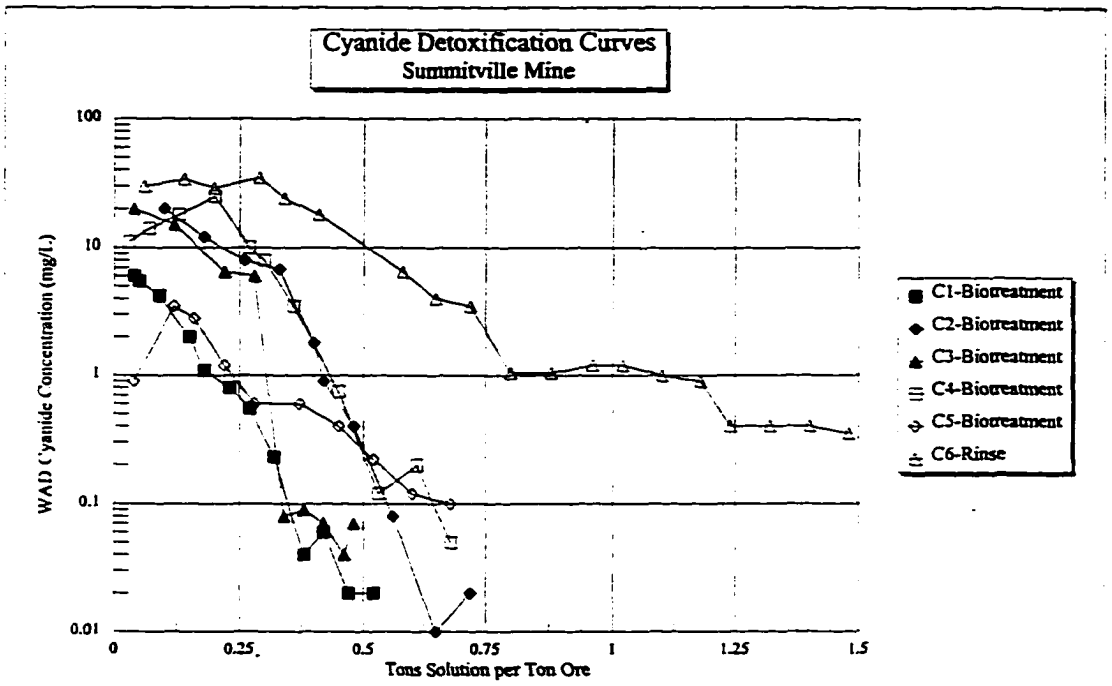


Figure 2.8 WAD cyanide detoxification curves for columns of ore from the Summitville mine (from Thompson, et al., 1995a)

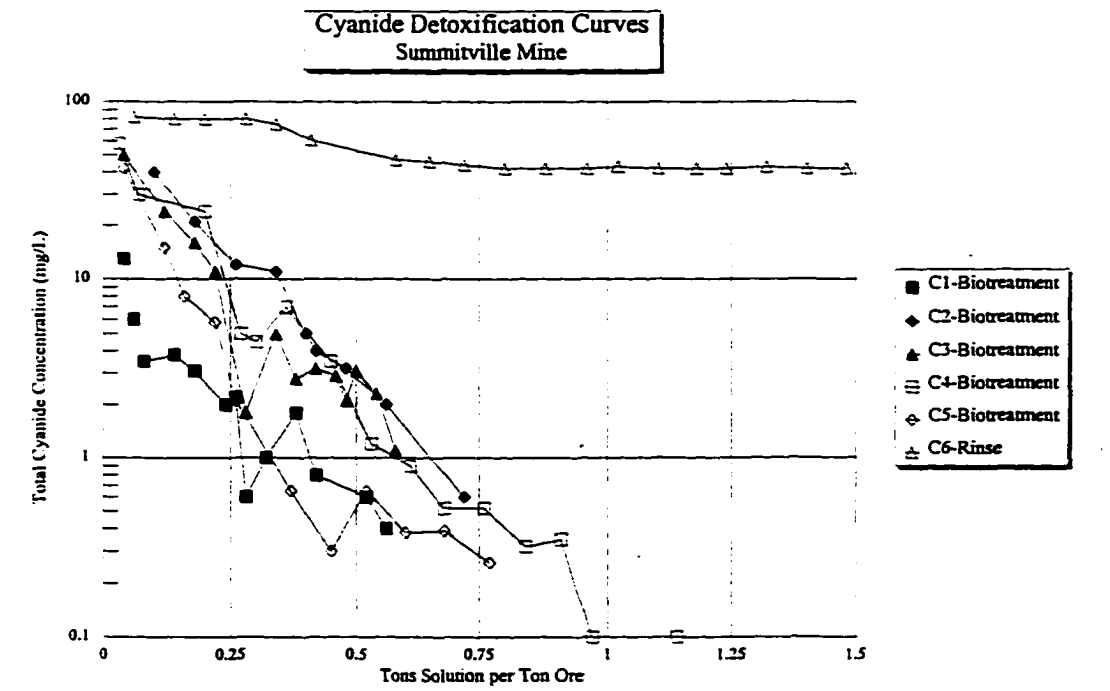


Figure 2.9 Total cyanide detoxification curves for columns of ore from the Summitville mine (from Thompson, et al., 1995a)

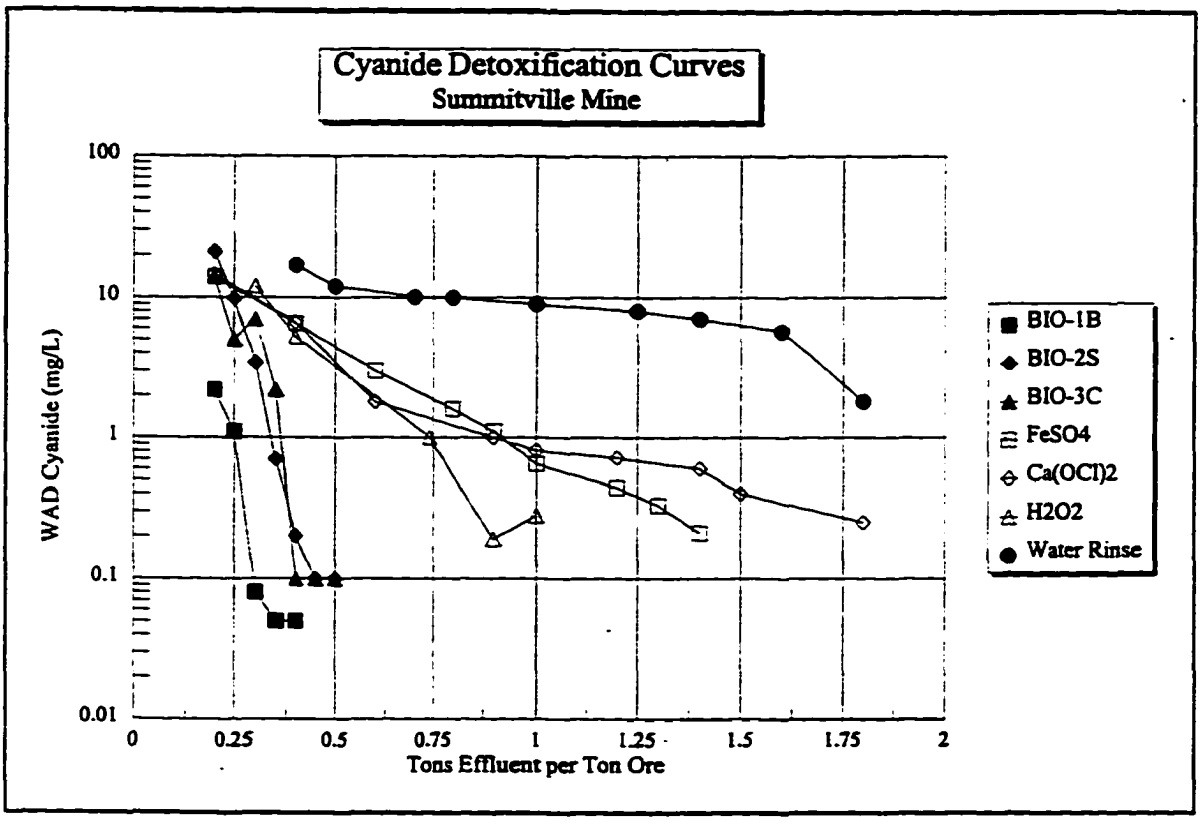


Figure 2.10 Comparison of cyanide detoxification methods (from Thompson, et al., 1995a).

This substantiates the claim that an in situ technique is far superior to an ex situ technique that requires the cyanide to be rinsed from the ore.

Another important discovery PSI made during the SITE testing was that the bacteria in the columns immobilized a portion of the soluble metals (Thompson, et al., 1995a; Thompson, et al., 1995b). During testing, PSI recorded a drop in copper concentrations from 30 mg/l to 0.2 mg/l in roughly two pore volumes. The metals were biomineralized onto the surface of the ore particles. This was an exciting discovery, especially if it could be applied in situ to heaps having high concentrations

of arsenic or other highly toxic heavy metals that become soluble while leaching. This would render the metals insoluble, reducing the cost of metal removal from the wastewater before final discharge.

Previous research by the U.S. Bureau of Mines Salt Lake Research Center, Leslie Thompson and Pintail Systems, Inc., and Homestake Mining Co. has proven the potential of biodetoxification of cyanide from spent heaps and mine wastewater. However, the tests described above were conducted in climates more temperate than Alaska. Little is known about the effects of the Alaskan climate on the performance of in situ biodetoxification of cyanide. Chapter 3 describes a series of tests undertaken to test the merit of this system in the Alaskan climate.

CHAPTER THREE

3.0 ALASKAN RESEARCH

One goal of this research was to assess bioaugmentation using microorganisms previously isolated at the USBM's Salt Lake Research Center (SLRC) and transported to Alaska. A second goal was to test native strains of microorganisms collected from an Alaskan mine site. Microorganisms from both sources were used for the treatment of waste from an Alaskan precious metals mine, in both laboratory and field conditions. Three tests were performed on each group of bacteria: cyanide degradation tests, winter survival rate tests, and an in situ heap detoxification test.

Another goal of this research was to develop conceptual designs for an in situ biological system to detoxify leached ore heaps. This was carried out and comparative analyses with other common in situ and ex situ heap detoxification processes were conducted and described in Chapter 4.

3.1 Testing Location

Fairbanks is located in Interior Alaska, as shown in Figure 3.1, and lies in the Tanana Valley watershed. Fairbanks has an average annual temperature of approximately 25° F

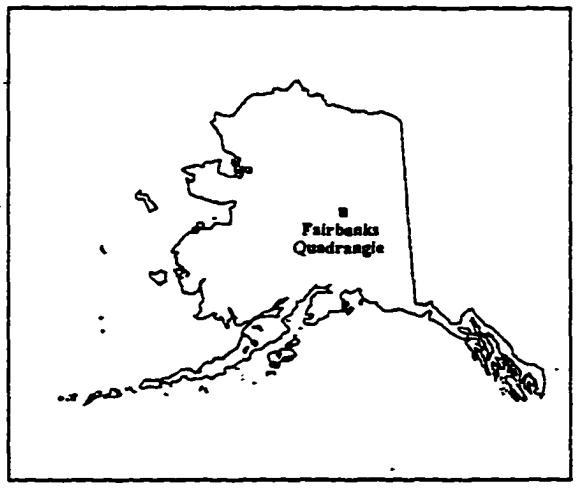


Figure 3.1 Location of the Fairbanks quadrangle.

(Hartman and Johnson, 1984). The topography rises on the north, east, and west sides of Fairbanks, creating a horseshoe shaped basin which is open to the south.

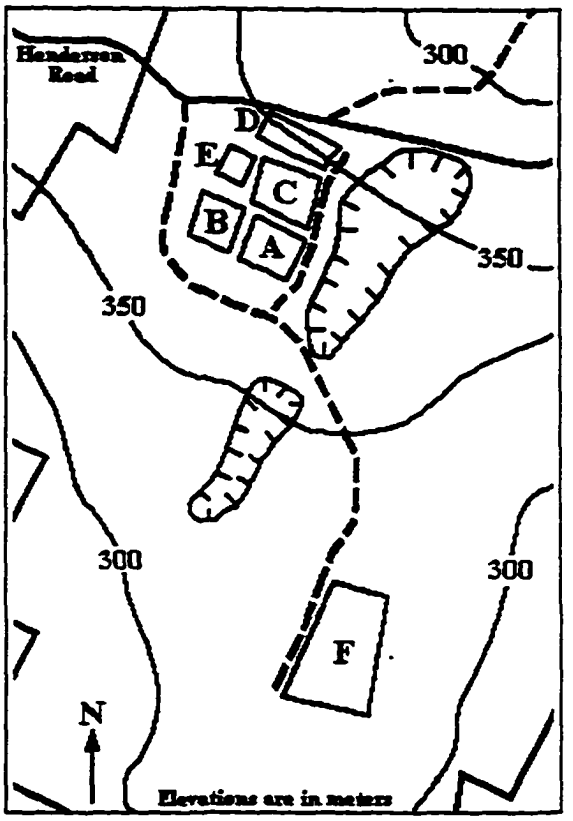


Figure 3.2 Partial map of the Ryan Lode site indicating the position of the heaps.

Due to the topography, cold temperatures settle into the Fairbanks basin, creating an inversion that holds cold air in the basin. These low temperatures are not readily displaced by warm weather fronts and temperatures can hover at -50° F for weeks at a time. However, the inversion layer is usually only about 500 to 1,000 feet thick, and there can be a 30° F difference between the temperature in low lying areas and the temperatures at the tops of the surrounding hills.

The average annual precipitation for Fairbanks is 11.2 inches and the average annual snowfall is 66.7 inches (Environmental Data Service, 1972). Because of the low annual temperature of Fairbanks, snow begins accumulating around October 15th and typically remains until April 15th, covering the ground for about six months of the year. Precipitation data for May-September 1994 were obtained from the National Weather Service office located at Fairbanks International Airport. The airport is

located about five miles southeast of the test site.

The field testing took place at the Ryan Lode mine site (Figure 3.2). The Ryan Lode site sits on the east side of Ester Dome at elevations ranging from 800 to 1,400 feet above mean sea level (MSL). About one third of the project sits on a south facing slope with an average grade of about 15%, one third sits on the ridge top, and the remaining one third sits on a north-facing slope with an average grade of about 20%.

Work by Santeford (1976) indicated a positive orographic (elevational) gradient in the annual rainfall in the Chena Valley. The calculated average multiplier for the Chena basin ranges was 1.7, with elevations ranging from 436 feet MSL (Fairbanks International Airport) to 4,000 feet MSL. R. Haugen, et al., (1982) reported the same orographic multiplier for a watershed approximately 30 miles northwest of Fairbanks. The elevations of the watershed in that study ranged from 689 to 2,710 feet MSL.

3.1.1 Site History

From 1987 to 1990, the Ryan Lode site was mined by Citigold Alaska, Inc. Citigold employed small-scale, open pit mining methods, with cyanide heap leaching used to recover gold values from the mined ore. During that period, Citigold constructed six heap leach pads: A, B, C, D, E, and F (see Figure 3.2). The only heap that has not yet been fully detoxified is heap F, which is scheduled to be finished during the summer of 1997. Removal of heavy metals from the mine wastewater is of special interest on Ester Dome, because the naturally occurring minerals in the area

cause high arsenic concentrations in most of the groundwater and well water.

Because of high snowfall during the winter of 1990-91, the mine management was forced to detoxify and discharge a portion of the water in the mine's pond system, so that the ponds could accommodate the water flowing off the heaps as the winter snows melted. The intention was to lower the cyanide content of the water to a safe level, and to remove any heavy metals from the water. The management chose to use calcium and sodium hypochlorite to lower the cyanide concentrations in the wastewater. These chemicals, which are similar to laundry bleach, are very effective in lowering cyanide concentration to about 10 parts per million. Below that level, their effectiveness decreases rapidly. The treatment program was not carefully monitored, so the extent to which these chemicals had been added was not known. Additional hypochlorite was added to pond water early in the summer of 1991, but was not effective in lowering the cyanide concentration.

In mid-summer 1991, the Ryan Lode site came under new management. The first task of the new site supervisors was to continue the treatment of mine wastewater, in order to have sufficient solution storage capacity for the upcoming winter. Rather than continuing the use of hypochlorite to treat the cyanide in the wastewater, they selected the INCO air-SO_2 process for treatment of the remaining wastewater. This process, which is patented by INCO, uses sulfur dioxide and oxygen, in the presence of a copper catalyst, to remove cyanide from solution. It has been shown to be effective in treating low concentration cyanide solutions at mining sites around the world, and was indeed effective in reducing the cyanide concentration

in Citigold's pond water to less than 0.5 parts per million, as required by a water discharge permit from the Alaska State Department of Environmental Conservation. As a part of the water treatment process administered by INCO, after the wastewater was treated by the air-SO_2 process, it was treated to precipitate the heavy metals it contained, notably arsenic, using an iron flocculant, and then discharged.

3.2 Collection and Isolation of Microorganisms

3.2.1 Experimental Work

Six agar slants were sent to UAF's Institute of Arctic Biology (IAB) from the SLRC. It was thought that the slants contained six strains of bacteria. However, three of the six agar slants were "contaminated" with other bacterial strains. The different bacterial colonies were isolated using standard colony isolation (three times) techniques on nutrient agar plates containing 20 mg/l sodium cyanide.

Once isolated cultures were obtained for the samples from the SLRC, the various bacterial colonies were inoculated into Dimethyl sulfoxide (DMSO) perms and placed into a -40° F freezer.

In addition to the agar slants from SLRC, samples of the water and agglomerate collected from the Ryan Lode site were tested in the lab at IAB. Cultures were grown from these samples to determine which organisms were present in the samples from the mine site. Standard colony isolation techniques were used to obtain a pure culture for each colony.

3.2.2 Experimental Results

Eleven, single-colony isolates were obtained from the six agar slants sent from the SLRC. From the water and agglomerate samples collected from Ryan Lode's operations, several microbial colonies were isolated as described above.

3.3 Cyanide Degradation Testing

3.3.1 Experimental Work

The eleven pure cultures isolated from the samples obtained from SLRC and the pure cultures isolated from the samples of water and agglomerate from the Ryan Lode site were tested to determine their ability to degrade or convert cyanide. The experiments described below are also published in Arps, et al., 1993.

3.3.2 Summary

In testing the bacterial samples obtained from the SLRC, only five of the eleven pure cultures successfully degraded cyanide from PGY medium. Of the five pure cultures that successfully degraded cyanide, the sample labeled UA7 (SLRC C1-I) performed the best in the lab testing, and was chosen for use in the field tests. Of the colonies isolated from the samples from the Ryan Lode property, many could tolerate cyanide concentrations up to 100 mg/l, but none were found to degrade or convert cyanide.

Several important questions were answered during this phase of work. First and foremost, it was concluded that the bacteria were degrading the cyanide

extracellularly, probably by producing an enzyme which oxidized the cyanide. It is not known how this strain of bacteria acquired the ability to oxidize cyanide, but there are several species of *Pseudomonas* that are cyanogens (cyanide producers). It is possible that this population of *Pseudomonas pseudoalcaligenes* may have grown in close proximity to cyanogens of the same genus and developed the ability to adapt an oxidase enzyme to oxidize cyanide. It could also be speculated that the use of this enzyme to oxidize cyanide is merely an accident, that the once the bacteria produced the enzyme, it attacked the cyanide preferentially.

3.3.2.1 Experiment 1

Experiment 1 entailed first streaking out the colonies on PGY agar plates containing potassium cyanide to bring the CN^- to 20 mg/l, and allowing them to grow for three days in an incubator at 27° C. Once sufficient growth had occurred on the plates, several culture tubes containing nutrient broth were inoculated. The culture tubes were grown overnight and pelletized by centrifuging. Several 125-ml side-arm flasks were prepared with 15 ml of PGY broth, inoculated with cells, and brought to a cyanide concentration of approximately 80 mg/l. The flasks were shaken gently on a shaker table, and 1.5 ml samples were removed as cell density increased. The cells in each 1.5 ml sample were again pelletized by centrifuging, and the supernatant was assayed for remaining cyanide using the micropicric acid method developed by Dr. Arps, which is a modification of the picric acid method provided in Appendix A.

3.3.2.2 Results from Experiment 1

The cyanide assays from Experiment 1 indicated that one of the organisms, UA7, was superior in its ability to decrease the cyanide levels in solution. However, oxidation of the cyanide by the air in the head space above the peptone, glycerol, and yeast extract (PGY) broth was suspect in this experiment, because the control samples of PGY broth showed corresponding lower cyanide levels with the samples inoculated with bacteria.

3.3.2.3 Experiment 2

Cells were removed from fresh PGY agar plates which contained 20 mg/l CN^- and inoculated into test tubes containing PGY broth. The samples were grown in a dark incubator to a Klett density of 90. The Klett-Summerson meter displays the drop in optical density of fluid using light at a wavelength of 540 nm. A Klett value of 90 represents approximately 4 X 10^8 bacterial cells per ml (Arps and Nelson, 1994).

For the first part of the experiment, cell suspensions were placed in screw-top test tubes, so that little air remained above the samples. Potassium cyanide was added to the tubes to bring the suspension to 100 mg/l CN^-. Aliquots were removed from the samples at various time points, and centrifuged to pellet the cells. The remaining liquid was assayed for cyanide using the micropicric method.

In the second part of the experiment, the remaining cell suspension, grown in the dark in an incubator to Klett 90, was shaken overnight in side-arm flasks, then centrifuged to pellet the cells, and filtered through a 0.2 μm filter to remove the cells.

Samples of the spent growth medium were brought to 100 mg/l cyanide; aliquots were taken at various time points and tested for their cyanide concentration.

3.3.2.4 Results from Experiment 2

During the experiment, the PGY broth and water controls maintained essentially the same WAD cyanide concentrations throughout the experiment, while the test tubes inoculated with the UA7 sample showed a dramatic drop in the cyanide concentration in a short time, as depicted in Figure 3.3. The drop in cyanide levels in the test tubes inoculated with UA7 was so dramatic that by the time the first set of aliquots was taken at time zero, the cyanide level had dropped to 2.5 mg/l. The test tubes which were inoculated with spent growth medium also showed a drop in cyanide

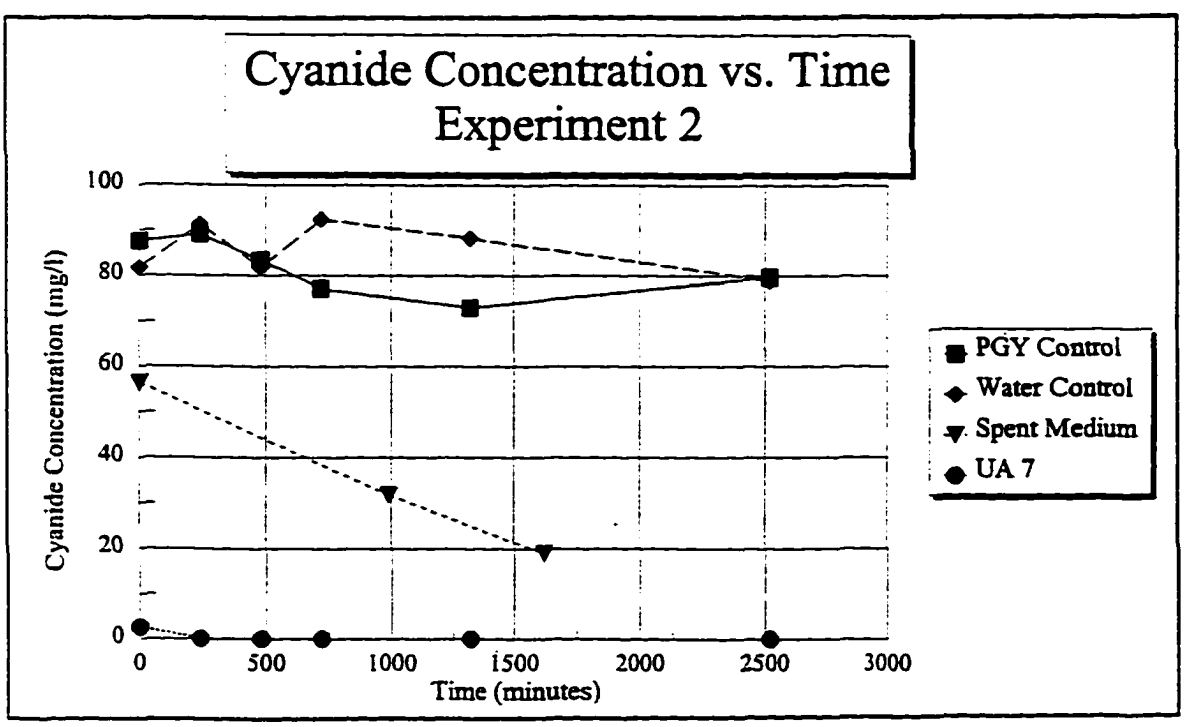


Figure 3.3 Results from Experiment 2.

concentration with time, but not to the same degree as the test tubes inoculated with the UA7 sample.

3.3.2.5 Experiment 3

This experiment was conducted nearly the same as Experiment 2. However, to slow the rapid conversion rates observed in Experiment 2, the CN^- was 200 mg/l, and half the concentration of bacterial cells used (Klett 45) for inoculation of the covered test tubes. The test tubes contained little air space, were kept in the dark, and not shaken. Aliquots were removed at various time points, and each aliquot was immediately placed in liquid nitrogen to stop all cell activity. The aliquots were then thawed quickly in a heat block at 90° C, centrifuged, and assayed for cyanide using the micropicric method.

3.3.2.6 Results from Experiment 3

The conditions in Experiment 3 did not markedly slow the oxidation of the cyanide in the test tubes inoculated with UA7, as shown in Figure 3.4. The test tubes inoculated with UA7 showed a reduction in the CN^- of 185 mg/l within a few minutes. Figure 3.4 also indicates the high and low cyanide concentration values for each aliquot. Note that the range for the UA7 sample was very small.

3.3.2.7 Experiment 4

This experiment was designed to determine if the mechanism of cyanide

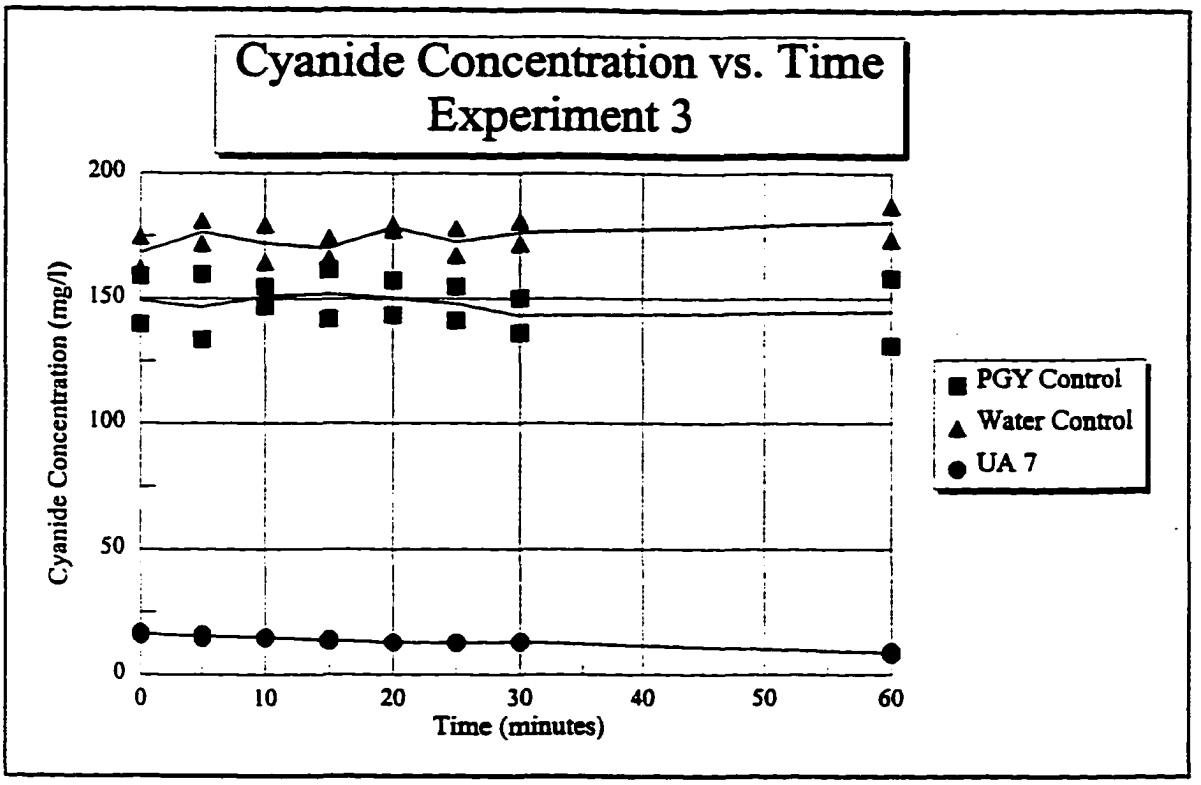


Figure 3.4 Results from Experiment 3.

conversion was chemical or biological. A chemical mechanism for cyanide conversion would be expected to show higher conversion rates at higher temperatures, while with a biological mechanism, the cyanide conversion rate would rise slightly with rising temperatures until the threshold value for bacterial life, approximately 40° C, was reached, after which the rate would decrease.

This experiment also tested the effects of high temperature on the ability of the bacterium to decrease cyanide concentration in solution. Using the same CN^- concentration (200 mg/l) as in Experiment 3, cyanide was added to heat-treated (3

minute boil) and untreated cell suspensions at Klett 90, and separate samples were incubated for one hour and overnight. Again, the cells were pelletized by centrifuging and the supernatant was assayed for remaining cyanide using the micropicric method.

3.3.2.8 Results from Experiment 4

Boiling clearly lowered the ability of the bacteria to oxidize cyanide (Figure 3.5), probably by damaging the cells. The temperature dependence of biological cyanide oxidation was also tested using the same cell suspension. Samples of cell suspensions were heated for several minutes at temperatures up to 60° C before addition of CN^- to 200 mg/l. The samples were incubated overnight, then centrifuged

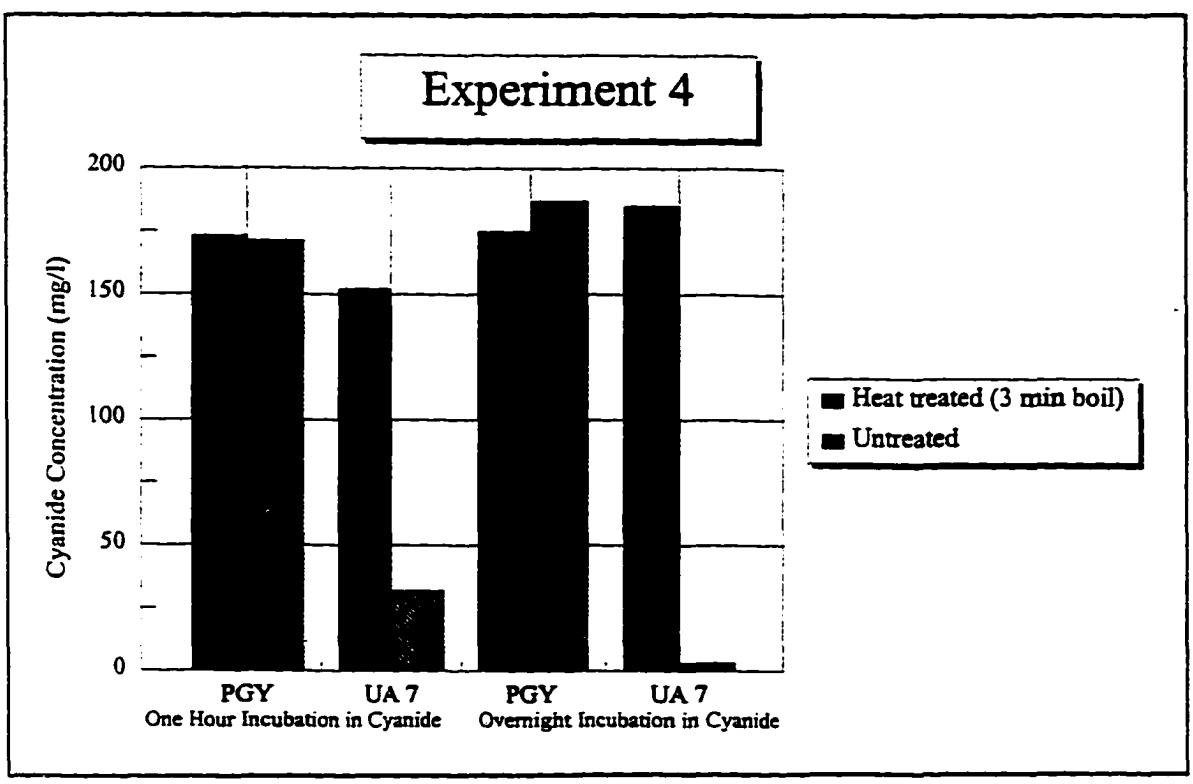


Figure 3.5 Effect of heat on cyanide degradation.

to pelletize the cells and assayed for cyanide. The ability of the cells to lower existing cyanide levels decreased markedly with increasing temperature, and continued to decrease with increasing time at a given temperature, as shown in Tables 3.1 and 3.2. This suggested that the conversion of cyanide in the samples was occurring through a biological mechanism and not a chemical mechanism.

Table 3.1 Cyanide remaining after cell treatment at various temperatures.

Temperature (° C)	WAD Cyanide Concentration	
	UA7	PGY Control
Ambient	124 mg/l	196 mg/l
37	131 mg/l	186 mg/l
60	179 mg/l	178 mg/l

Table 3.2 Cyanide remaining after cell treatment at 60° C.

Time (minutes)	WAD Cyanide Remaining (mg/l)
2	152
5	146
10	179

3.3.2.9 Experiment 5

This experiment was designed to determine if the biological cyanide conversion

mechanism was intracellular or extracellular. Bacteria were transferred from agar plates containing PGY and 20 mg/l CN^- and grown to high cell density (Klett 275) in PGY broth. After being stored on ice overnight, the cells were lysed using a sonicator, then pelletized by centrifugation. Increasing volumes of supernatant were tested for the ability to lower cyanide levels and compared to similarly increasing volumes of unsonicated cells at Klett 275. One set of cells were stored overnight on ice and compared to cells taken directly from the plates (fresh cells). All samples were analyzed using the micropicric method.

3.3.2.10 Results from Experiment 5

Fresh cells clearly reduced the CN^- concentration (Figure 3.6) better than both the cells stored overnight on ice and the cell extract obtained from the sonicated cells. An important result of this experiment was that an addition of only 0.01 ml (10 µL) of fresh cells at Klett 275 reduced the cyanide concentration by 25 mg/l.

3.3.2.11 Experiment 6

Experiment 6 tested the ability of resting cells to degrade high concentrations of cyanide. The test strain was grown for 40 hours to an Klett density of 134 in PGY broth which contained no additional cyanide. The broth was centrifuged to pelletize the cells and resuspended in 26 ml of assay buffer containing 0.4 M sodium chloride, 50 mM sodium phosphate, and 0.01% gelatin (pH 7.0). The cells were recentrifuged and resuspended in the same buffer to a high cell density (optical density of 24.7 at

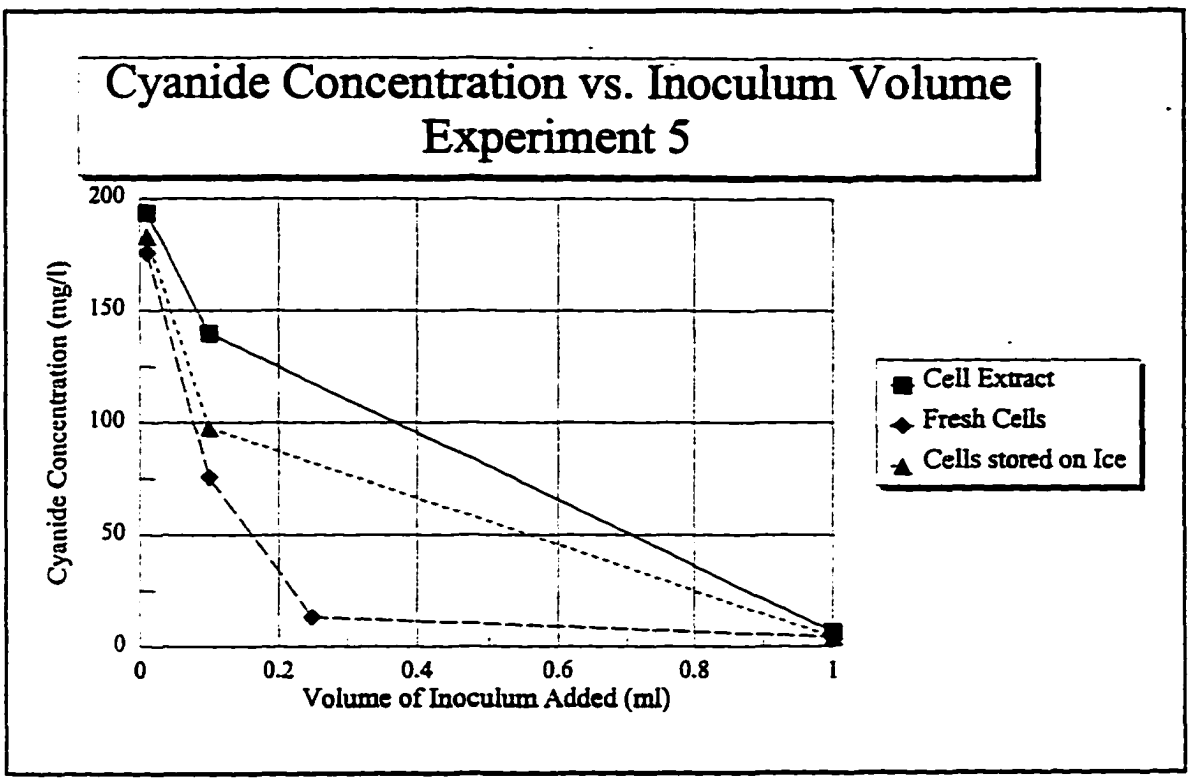


Figure 3.6 Results from Experiment 5.

540 nm). Aliquots of 1.2 ml were added to serum vials and potassium cyanide was added at increasing concentrations of 90 to 1,800 mg/l CN^-. After 12 hours, the samples were centrifuged and the supernatant assayed for cyanide using the micropicric method.

3.3.2.12 Results from Experiment 6

Isolate UA4/7 was able to convert CN^- provided at concentrations as high as 900 mg/l (Table 3.3). However, at concentrations of 1,800 mg/l, the amount of the CN^-degraded was significantly reduced, and the presence of a slimy mucous layer

covering the cells was observed.

Table 3.3 Results from Experiment 6.

Initial WAD Cyanide Concentration (mg/l)	Remaining WAD Cyanide Concentration (mg/l)		
	UA7	% Degraded	PGY Control
90	0	100	71
180	0	100	140
450	0	100	355
900	0	100	710
1800	1240	11	1400

3.4 Identification of Microorganisms

3.4.1 Experimental Work

The identification of the bacterial strain received from the SLRC was completed by Karen Dohrman, of Microbial I.D., Inc. in Newark, Delaware using gas chromatography (GC) of the cellular fatty acids. The company operates an automated Microbial Identification System which compares the GC pattern of cellular fatty acids to a database with over 60,000 analyses of different strains of bacteria. A detailed description of the experimental methods used in identification can be found in Appendix D.

3.4.2 Experimental Results

Five pure cultures isolated at UAF, as described in section 3.2, were sent to Microbial I.D., Inc. for fatty acid analysis. The results showed that there were only three separate organisms present in the six samples. Isolates UA7, UA16, and UA19 were identified as *Pseudomonas pseudoalcaligenes*, isolate UA10 was identified as *Pseudomonas diminuntia*, and UA1 was an uncataloged, gram-positive bacterium.

The GC analysis indicated that there was a 85.4% correlation between the GC outputs of the type strain of *P. pseudoalcaligenes* and the USBM GS-OP sample. The data output from the fatty acid analyses are shown in Appendix E. Figure 3.7 is a photomicrograph of *P. pseudoalcaligenes* taken by a scanning electron microscope.

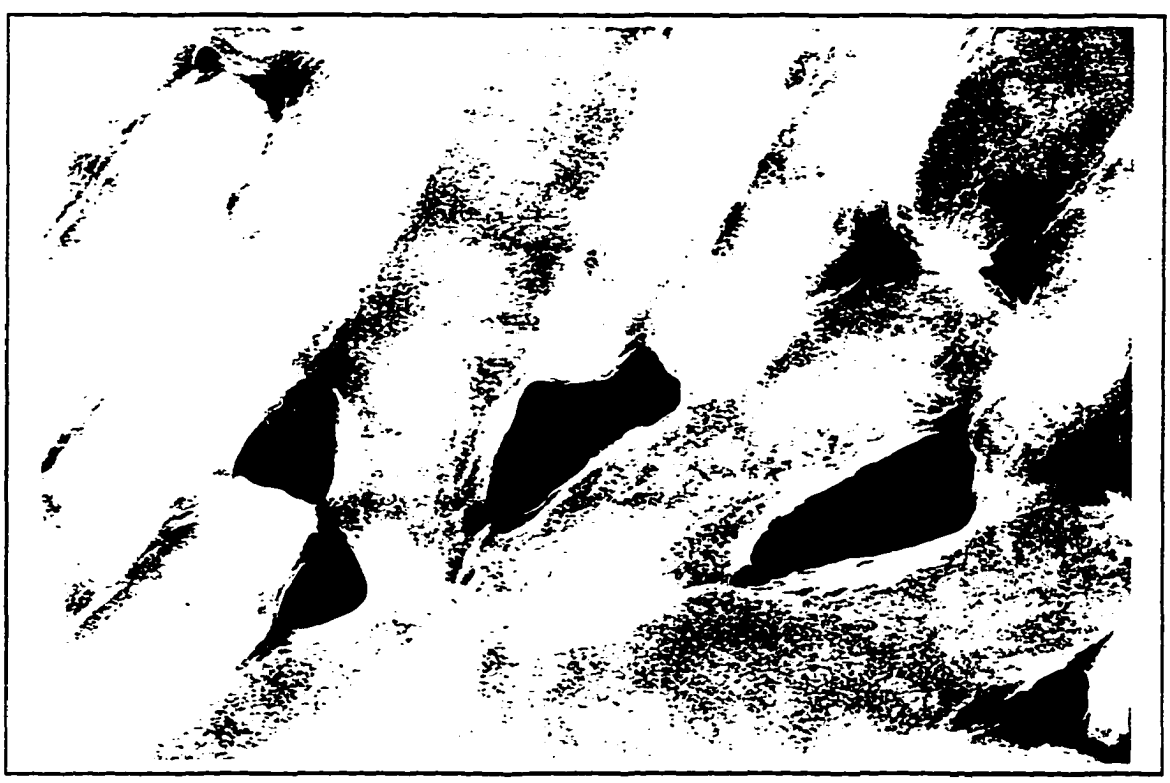


Figure 3.7 Photomicrograph of *Pseudomonas pseudoalcaligenes* from a scanning electron microscope (not to scale).

The genus *Pseudomonas* is a group containing straight or slightly curved rods with polar flagella that are always motile. They are heterotrophic (organisms which use organic energy sources), aerobes (organisms which function in the presence of oxygen), never show a fermentative metabolism, and are almost always oxidase positive (Brock and Madigan, 1988).

3.5 Winter Survival Testing

To assess the viability of using biological cyanide detoxification in the Alaskan environment, a field experiment to test the survivability of the bacteria in a heap was conducted during the winter of 1992-93. The experiment involved placing samples of inoculated ore in specially constructed boxes into heap F at the Ryan Lode site.

3.5.1 Winter Survival Experimental Setup

Eight, one-cubic-foot boxes were constructed using spruce 2x2's and galvanized 1/4 inch hardware cloth (screen). Five screen bags containing agglomerate from heap F were placed in each box. After the five bags were stacked in each box, the remaining area around the bags was filled with agglomerate and the boxes were stapled shut. The boxes were then buried in the side of heap F on October 13, 1992 and allowed to "winter over" until May 15, 1993.

The procedure for inoculating the bags was as follows:

UA7 was streaked out on nutrient agar (NA) plates containing 20 mg/l sodium cyanide. Once there was sufficient growth on the plates, two-liter flasks containing

nutrient broth were inoculated with bacteria from the NA plates. The two-liter flasks were placed on shakers and grown at either room temperature or 27° C, depending on the shaker used.

Once sufficient growth had occurred in the flasks, the inoculum was poured into large Rubbermaid containers. 100 ml of inoculum were removed and placed on ice to be used for population estimation using the Klett meter.

Forty, 6"x10" bags were constructed from standard, plastic-coated, fiberglass window screen using a sewing machine. Approximately 500 grams of agglomerate were placed in each bag. The bags labeled number one in each box, for a total of eight bags, were not inoculated with any bacteria. Once filled with agglomerate, they were stapled shut and set aside to be placed in the boxes later.

After being filled with agglomerate, the bags labeled numbers three and five in each box, for a total of 16 bags, were stapled shut and autoclaved to kill any organisms present in the agglomerate. Once autoclaved, the 16 bags were placed into plastic milk crates and submerged in the Rubbermaid containers for 12 minutes, then removed and allowed to drain for several minutes. Once drained, the 16 screen bags were sealed inside larger Gore-Tex bags. The purpose of the Gore-Tex bags, which have a nominal pore size less than 1 micron, was to allow moisture and possibly nutrients and cyanide to pass into the agglomerate while not permitting the inoculated bacteria to leave the agglomerate in the bags or other bacteria to enter. This was done to ensure that when the bacterial counts for the agglomerate in each bag were made, the only bacteria counted would be UA7.

The eight screen bags labeled number four in each box were placed in the plastic milk crates and submerged in the inoculum for 12 minutes. Again, after submersion in the inoculum, the eight bags were allowed to drain for several minutes.

Before the remaining eight bags, labeled number two in each box, were submerged in the inoculum, the Rubbermaid container used for inoculation was emptied into a glass, 50-liter carboy and autoclaved. This killed the population of UA7 in the inoculum, but left most of the nutrients in the inoculum. After being autoclaved and allowed to cool, the inoculum was poured back into the sterilized Rubbermaid container. The remaining eight bags were placed in the sterilized plastic milk crate and submerged for 12 minutes. After the 12 minutes of submersion, the milk crate was removed and the bags were allowed to drain for several minutes.

Table 3.4 indicates the treatment for the five bags in each box and the order in which the bags were stacked in the eight boxes.

Table 3.4 Treatments for the over-winter samples.

Bag Number	Treatment
1	Inoculated with sterile PGY medium.
2	Inoculated with heat killed UA7.
3	Autoclaved, inoculated with UA7, and placed in Gore-Tex bags.
4	Inoculated with UA7.
5	Autoclaved, inoculated with UA7, and placed in Gore-Tex bags.

After the five bags were stacked in each box, the remaining area around the

bags was filled with agglomerate and the remaining sides of the boxes were stapled shut. The boxes were transported to the site and buried in heap F as shown in Figure 3.8. Photograph 3.1 was taken as the boxes were buried shows the bottom of the excavation and boxes seven (foreground) and 8 before the second layer of boxes was placed in the trench. Photograph 3.2 shows boxes two (foreground), three, and four (background) before the excavation was backfilled.

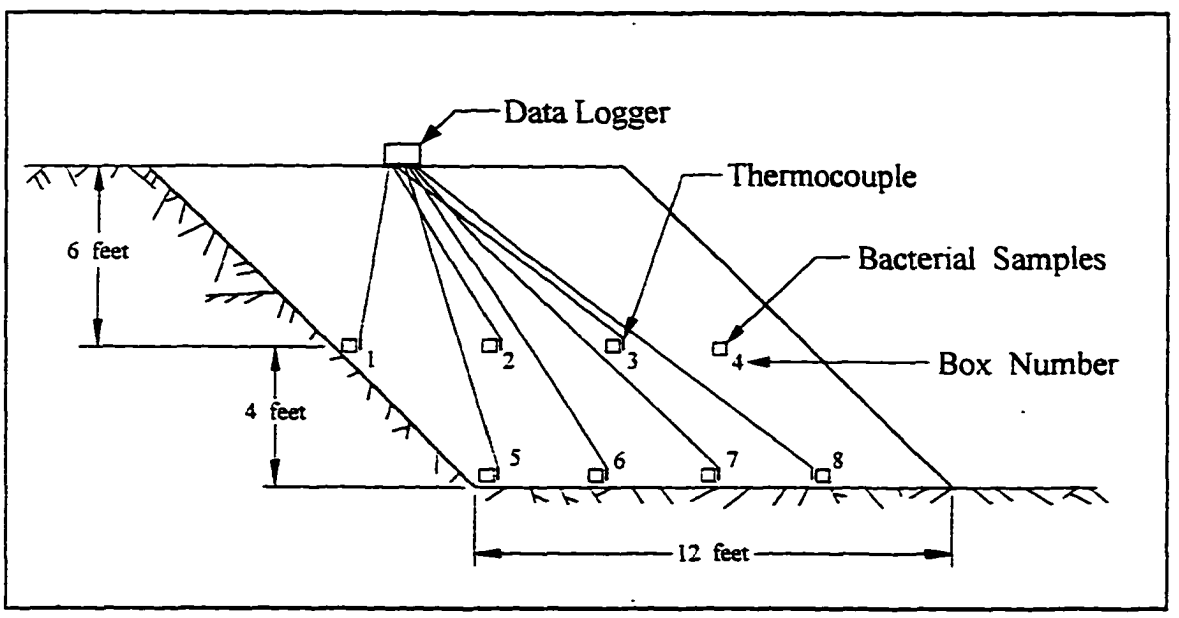


Figure 3.8 Location of the boxes during the winter survival test.

A length of K type thermocouple wire (Chromel-Alumel) was placed beside each box, except for box four. The thermocouple wires were connected to a Campbell Scientific 21XL datalogger, and the temperatures at each box and the ambient temperature at the datalogger on the heap surface were recorded hourly. About once a month the data stored in the datalogger were downloaded to a notebook computer in

Photograph 3.1 The bottom of the excavation and boxes 7 (foreground) and 8.

Photograph 3.2 Boxes 2 (foreground), 3, and 4 (background) before the excavation was backfilled.

the field and taken back to the University for reduction.

3.5.2 Results from Winter Survival Experiment

The original plan was to remove boxes at various times during the winter of 1992-93 to estimate the survival rate of the inoculated bacteria over the winter. This plan could not be carried out because of the depth of freeze in the heap, so all the boxes were removed together on May 15, 1993. The boxes were over-packed with agglomerate in large plastic tubs, the tops were sealed with plastic, and placed in a coldroom (40° F) at IAB. On October 1, 1993, the bags were removed from the boxes in the coldroom and three subsamples from each bag were sent to Elaine Ingham in Portland, Oregon for total and active bacterial population counts using separate staining techniques for the total and active populations. In addition to the subsamples sent to Portland, subsamples were removed from the bags and inoculated onto PGY plates containing 200 mg/l CN^- to test the soil for cyanide oxidizing bacteria.

It is unfortunate that the boxes containing the over-wintered bacteria were allowed to sit in the coldroom at IAB as long as they did before they were sent out for population counts. However, even after six months in the coldroom, the population counts indicated that on average, 5-20% of the bacterial population was still active. The data from the bacterial count testing are provided in Table 3.5 and the average active biomass for the bags in each bag is plotted in Figure 3.9. The total bacterial count is plotted in Figure 3.10. It is interesting to note in Figure 3.10, that for the bags numbered one in each box, that were untreated samples, the total bacterial

Table 3.5 Data from bacterial count tests.

Box/Bag	Sample	Total Bacteria #/GDW	Total Biomass ug/GDW	Active/total Biomass	Box/Bag	Sample	Total Bacteria #/GDW	Total Biomass ug/GDW	Active/total Biomass
01/01	A	2.90E+07	5.790	0.116	05/01	A	4.27E+07	8.540	0.096
	B	2.09E+07	4.170	0.260		B	1.37E+07	3.340	0.357
	C	2.15E+07	4.310	0.312		C	3.09E+07	6.170	0.160
01/02	A	2.42E+07	4.840	0.163	05/02	A	6.03E+07	12.050	0.121
	B	8.09E+06	1.620	0.233		B	3.67E+07	7.340	0.172
	C	5.80E+06	1.160	0.259		C	3.16E+07	6.310	0.063
01/03	A	9.40E+06	1.880	0.106	05/03	A	4.46E+07	8.920	0.221
	B	1.31E+07	2.630	0.119		B	3.72E+07	7.450	0.245
	C	1.39E+07	2.790	0.029		C	8.15E+07	16.300	0.083
01/04	A	3.12E+07	6.230	0.048	05/04	A	5.30E+07	10.600	0.061
	B	1.87E+07	3.740	0.133		B	3.26E+07	6.520	0.111
	C	1.95E+07	3.900	0.145		C	3.47E+07	6.950	0.062
01/05	A	2.17E+07	4.330	0.046	05/05	A	5.70E+07	11.390	0.043
	B	8.75E+06	1.750	0.113		B	5.71E+07	11.410	0.046
	C	1.32E+07	2.640	0.089		C	4.25E+07	8.510	0.053
02/01	A	2.30E+07	4.600	0.057	06/01	A	5.48E+06	1.100	0.032
	B	2.43E+07	4.870	0.085		B	7.87E+06	1.570	0.113
	C	2.40E+07	4.790	0.047		C	1.35E+07	2.710	0.000
02/02	A	2.62E+07	5.240	0.064	06/02	A	1.12E+07	2.240	0.032
	B	3.24E+07	6.470	0.057		B	9.07E+06	1.810	0.078
	C	4.99E+06	1.000	0.261		C	1.29E+07	2.590	0.014
02/03	A	5.23E+06	1.050	0.075	06/03	A	6.10E+06	1.220	0.119
	B	3.13E+06	0.630	0.000		B	5.61E+06	1.120	0.065
	C	5.33E+06	1.070	0.186		C	8.50E+06	1.700	0.085
02/04	A	9.40E+06	1.880	0.078	06/04	A	1.22E+07	2.440	0.380
	B	8.96E+06	1.790	0.041		B	5.96E+06	1.190	0.149
	C	9.22E+06	1.840	0.040		C	7.20E+06	1.440	0.199
02/05	A	9.24E+06	1.850	0.277	06/05	A	1.23E+07	2.450	0.088
	B	6.99E+06	1.400	0.315		B	1.15E+07	2.300	0.109
	C	1.80E+07	3.600	0.164		C	1.97E+07	3.940	0.072
03/01	A	1.72E+07	3.450	0.064	07/01	A	9.55E+06	1.910	0.078
	B	1.39E+06	2.780	0.013		B	7.31E+06	1.460	0.026
	C	3.98E+06	1.400	0.027		C	1.25E+07	2.510	0.030
03/02	A	1.58E+07	3.150	0.012	07/02	A	1.01E+07	2.020	0.186
	B	2.82E+07	5.640	0.000		B	9.08E+06	1.820	0.104
	C	9.68E+06	1.940	0.019		C	6.80E+06	1.360	0.138
03/03	A	7.55E+06	1.510	0.027	07/03	A	3.15E+07	6.300	0.051
	B	2.53E+07	5.060	0.061		B	4.41E+07	8.820	0.009
	C	8.51E+06	1.700	0.233		C	2.67E+07	5.340	0.015
03/04	A	2.71E+06	0.540	0.068	07/04	A	2.59E+07	5.180	0.043
	B	5.44E+06	1.090	0.068		B	1.18E+07	2.350	0.174
	C	3.48E+06	0.700	0.160		C	1.28E+07	2.570	0.117
03/05	A	1.67E+07	3.340	0.086	07/05	A	3.29E+07	6.570	0.081
	B	1.45E+07	2.910	0.186		B	3.44E+07	6.880	0.059
	C	1.52E+07	3.030	0.027		C	2.67E+07	5.340	0.091
04/01	A	1.64E+07	3.280	0.057	08/01	A	8.93E+06	1.790	0.393
	B	6.58E+06	1.320	0.401		B	1.34E+07	5.680	0.193
	C	3.79E+06	0.600	0.447		C	1.07E+07	2.140	0.064

Table 3.5 continued.

Box/Bag	Sample	Total Bacteria #/GDW	Total Biomass ug/GDW	Active/total Biomass	Box/Bag	Sample	Total Bacteria #/GDW	Total Biomass ug/GDW	Active/total Biomass
04/02	A	2.98E+06	1.140	0.435	08/02	A	5.45E+07	10.910	0.215
	B	5.71E+06	1.840	0.130		B	1.03E+07	2.060	0.284
	C	9.18E+06	1.580	0.081		C	5.47E+07	10.940	0.172
04/03	A	7.90E+06	3.920	0.026	08/03	A	1.42E+07	2.840	0.225
	B	1.96E+07	1.350	0.082		B	1.49E+07	2.980	0.203
	C	6.77E+06	0.096	0.149		C	1.00E+07	2.010	0.322
04/04	A	2.14E+07	4.290	0.096	08/04	A	7.94E+06	1.590	0.047
	B	1.27E+07	2.530	0.148		B	1.09E+07	2.170	0.186
	C	1.32E+07	2.650	0.295		C	7.18E+06	1.440	0.180
04/05	A	1.12E+07	2.240	0.037	08/05	A	8.21E+06	1.640	0.409
	B	1.42E+06	0.280	0.000		B	6.35E+06	1.270	0.466
	C	1.15E+06	0.230	0.177		C	4.97E+06	0.990	0.785

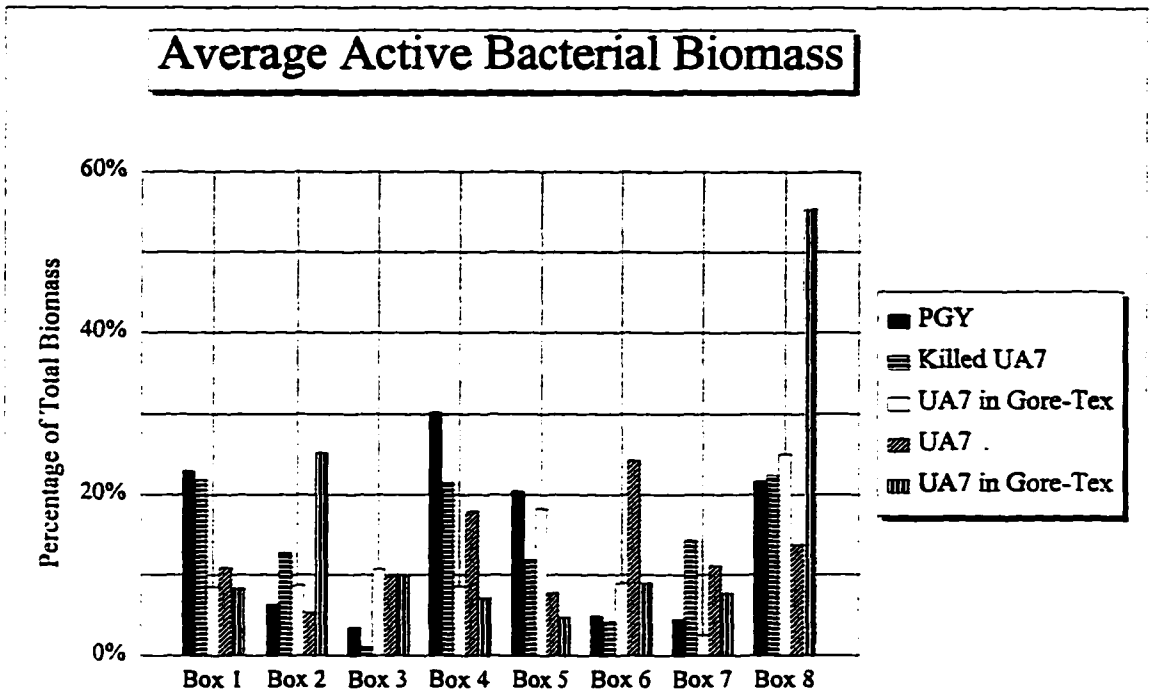


Figure 3.9 Average active bacterial biomass for each box.

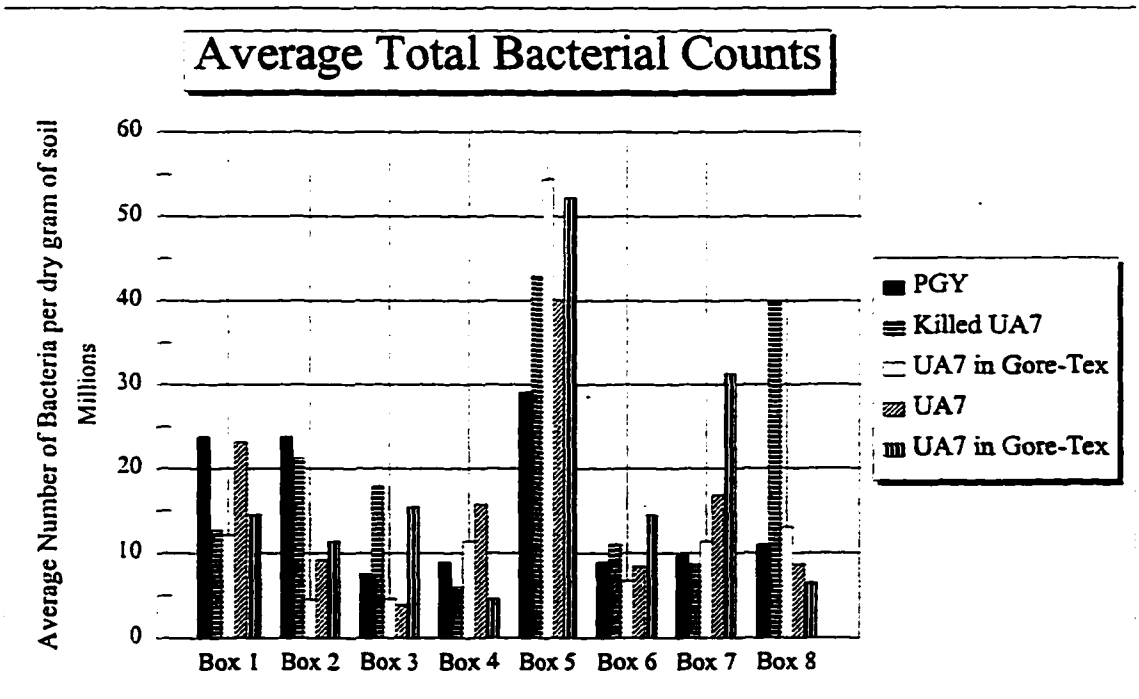


Figure 3.10 Average total bacterial counts for each box.

populations were nearly the same as the bacterial populations in bags that were inoculated with bacteria. This suggests that the bacteria colonized the uninoculated agglomerate sometime between inoculation and counting. The data in Figure 3.10 also shows that the total number of bacteria in Box five was approximately two-and-a-half times higher than the population in the next highest box. It is unlikely that the initial population of bacteria inoculated into the bags varied a great deal, so the bacteria in Box five may have continued to actively grow and consume the nutrient medium directly after placement, resulting in larger population counts in the spring. This could be due to the fact that Box five was buried the deepest and furthest from the side slope of the heap, as depicted in Figure 3.8. As would be expected, Boxes three and four had the lowest total bacterial populations, because they were the closest to the side and top of the heap. The total bacterial population in Box three was only half the average total bacterial population of all the boxes.

The bacterial populations reported for bags numbered three and five in each box are also important, because these samples were sealed inside the Gore-Tex bags which allowed the migration of water, but not bacteria, in and out of the samples. No discernable differences could be found when analyzing the data for these two bags. This would suggest that the bacteria inoculated into the heap could survive over the winter, and that competition from native organisms would not be a substantial problem.

Once sufficient growth was established on the PGY plates containing 200 mg/l CN^-, the colonies were isolated and tested for cyanide degradation. None of the

colonies tested showed an ability to degrade cyanide. However, the cyanide-degrader (UA7) used in this study was in the presence of only very small quantities of cyanide (3.68 mg/kg dry ore) in the heap and in the coldroom. This cyanide concentration may not have been sufficient for maintaining the capacity to degrade cyanide, since cyanide degradation has shown in other organisms to require induction (Ingvorsen, et al., 1991).

The temperature recording system performed fairly well throughout the 1992-93 winter, but some data loss occurred from equipment malfunctions due to extreme temperatures. Figure 3.11 is a partial curve indicating the estimated snow cover, ambient temperatures, and temperatures at each box during the winter. Table 3.6 lists the temperatures recorded at each box during the winter of 1992-93 as well as estimated snow depths on heap F.

As expected, the temperatures inside the heap corresponded very well to the amount of snow cover on the heap and also displayed the classic temperature lag at depth in response to temperature changes at the heap surface. Snow cover on the heap was difficult to quantify due to a windy period at the mine site during the second week of January, 1993. The wind blew the snow off the top of the heap and deposited large amounts on the side slope and in the depression adjacent to the containment berm. Because of this snow drifting, there was minimal snow cover on top of the heap, and approximately four feet of snow on the lower portions of the heap that acted as insulation over the winter.

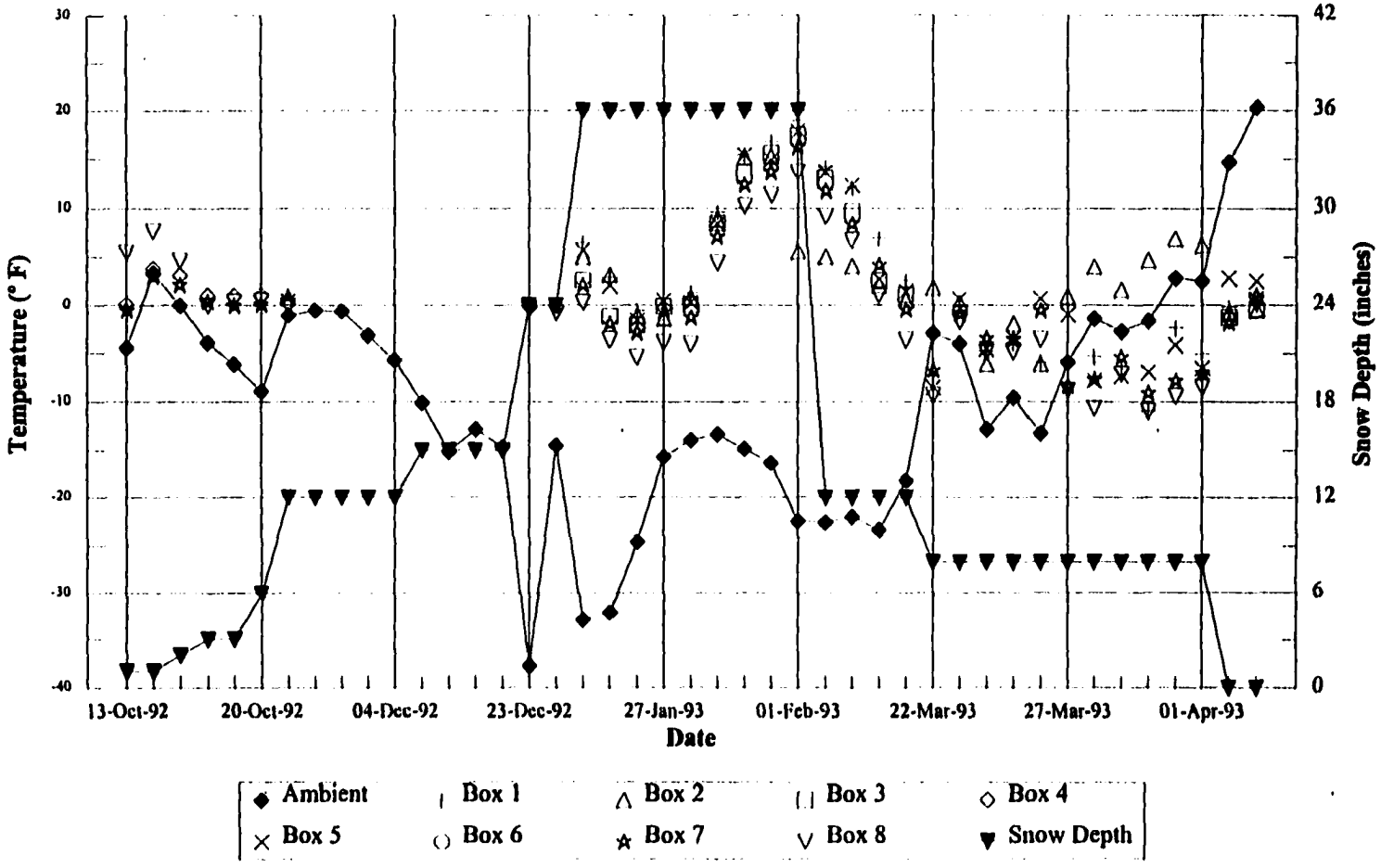


Figure 3.11 Temperatures in heap F during the 1992-93 winter.

Table 3.6 Temperatures in heap F during the 1992-93 winter.

			Inches of	Temperature (° F)							
Date	Day #	Time	Snow*	Ambient	Box 1	Box 2	Box 3	Box 4	Box 5	Box 6	Box 7	Box 8
13-Oct-92	0	04:30 PM	1	-4.4				0.1			-0.5	5.4
14-Oct-92	1	02:10 PM	1	3.3				3.8			3.1	7.7
15-Oct-92	2	04:00 PM	2	0.0				3.1			2.2	4.6
16-Oct-92	3	03:45 PM	3	-3.9				1.0			0.4	0.0
17-Oct-92	4	02:20 PM	3	-6.1				0.9			0.0	0.3
20-Oct-92	7	04:00 PM	6	-8.9				1.0			0.1	0.4
06-Nov-92	24	02:30 PM	12	-1.1				0.2			0.9	-0.1
01-Dec-92	49	04:13 PM	12	-0.6								
02-Dec-92	50	12:14 PM	12	-0.6								
03-Dec-92	51	12:02 PM	12	-3.2								
04-Dec-92	52	01:19 PM	12	-5.7								
05-Dec-92	53	04:00 AM	15	-10.1								
06-Dec-92	54	11:00 AM	15	-15.1								
07-Dec-92	55	11:00 AM	15	-12.8								
08-Dec-92	56	11:00 AM	15	-14.6								
23-Dec-92	71	12:00 PM	24	-37.8				-0.2				-0.4
10-Jan-93	89	12:00 PM	24	-14.4				-0.1				-0.8
24-Jan-93	103	09:00 PM	36	-33.0	6.4	5.0	2.6		5.6	2.9	2.1	0.3
25-Jan-93	104	12:00 PM	36	-32.2	2.8	3.1	-1.2		2.0	-2.4	-1.9	-3.6
26-Jan-93	105	12:00 PM	36	-24.6	-0.6	-1.2	-2.2		-1.7	-2.6	-2.8	-5.4
27-Jan-93	106	10:00 PM	36	-15.7	-2.1	-1.5	-0.2		0.5	-0.7	-0.7	-3.9
28-Jan-93	107	11:00 AM	36	-14.0	1.3	0.7	-0.1		0.2	-0.3	-1.3	-4.1
29-Jan-93	108	07:00 PM	36	-13.3	9.6	8.8	8.4		8.4	8.3	7.1	4.4
30-Jan-93	109	09:00 AM	36	-14.8	15.4	15.2	13.5		15.4	13.0	12.4	10.3
31-Jan-93	110	06:00 AM	36	-16.3	16.8	15.3	15.6		14.9	15.5	13.7	11.4
01-Feb-93	111	12:00 PM	36	-22.4	18.9	5.6	17.4		17.9	17.1	16.3	13.6
02-Feb-93	112	11:00 AM	12	-22.6	14.2	5.0	13.0		13.7	13.4	11.8	9.2
03-Feb-93	113	02:00 PM	12	-22.0	12.1	4.1	9.7		12.3	9.7	8.4	6.7
04-Feb-93	114	11:00 AM	12	-23.3	6.9	2.0	2.5		2.6	4.7	4.2	1.0
05-Feb-93	115	12:00 PM	12	-18.2	2.5	0.6	1.1		1.4	2.0	-0.5	-3.6
22-Mar-93	160	12:00 PM	8	-2.9	-7.3	1.8			-8.5	-8.9	-6.7	-9.3
23-Mar-93	161	12:00 PM	8	-4.0	-0.2	0.2			0.7	0.5	-0.9	-1.7
24-Mar-93	162	12:00 PM	8	-12.8	-4.8	-6.1			-4.6	-4.1	-3.4	-4.9
25-Mar-93	163	12:00 PM	8	-9.5	-3.9	-1.9			-3.4	-3.1	-3.5	-4.8
26-Mar-93	164	01:00 PM	8	-13.2	-5.9	-6.0			0.7	-2.0	-0.6	-3.6
27-Mar-93	165	12:00 PM	8	-5.8	-5.6	1.0			-1.0	-6.9	-8.3	-8.7
28-Mar-93	166	01:00 PM	8	-1.4	-5.3	4.0			-7.7	-8.5	-7.6	-10.6
29-Mar-93	167	10:00 AM	8	-2.7	-6.3	1.6			-7.3	-8.2	-5.3	-7.1
30-Mar-93	168	12:00 PM	8	-1.7	-10.9	4.7			-6.9	0.4	-9.0	-11.0
31-Mar-93	169	05:00 PM	8	2.8	-2.4	6.9			-4.1	-6.4	-7.8	-9.4
01-Apr-93	170	12:00 PM	8	2.5	-5.0	6.2			-6.5	-6.6	-7.0	-8.5
14-May-93	213	12:00 PM	0	14.7	-0.2	-0.9	-1.4		2.8	-1.4	-1.8	-1.7
15-May-93	214	12:00 PM	0	20.4	0.1	-0.5	-0.4		2.5	1.0	1.1	0.2

* - The snow depths were visual estimations.

26-Jun-95

3.6 In situ Heap Testing

When the project was first proposed to the Alaska Science and Technology Foundation by Drs. Nelson and Arps, it was envisioned that a series of column tests would be conducted to assess the abilities of biological cyanide detoxification in the Alaskan environment. When the opportunity to conduct a field test at the Ryan Lode site presented itself, the research plans were changed, and the test heap was substituted for the laboratory column tests. They felt that if a field test was conducted properly, it could yield better information about the process than laboratory column tests. The decision was made in June 1993 to conduct a field heap test, and work was begun to carry out this plan.

3.6.1 Test Heap Experimental Setup

During August 1993, a test heap of approximately 180 metric tons of agglomerate from the existing F heap and an adjacent test pond were constructed in order to simulate a working heap. A berm was constructed around the bottom of the test heap to contain the agglomerate and leachate. A notch was left in the berm between the test heap and pond to allow the test heap to drain freely into the test pond. Figure 3.12 depicts the solution cycle for the test heap.

Five, 6.7 m wide sheets of Gundle 40 mil High Density Polyethylene (HDPE) liner were used to separate the test heap and test pond from the existing heap F. Three sheets of liner were welded together and draped over the top tier of the current heap F. Approximately 60 m of 7.6 cm perforated plastic drain tile was placed

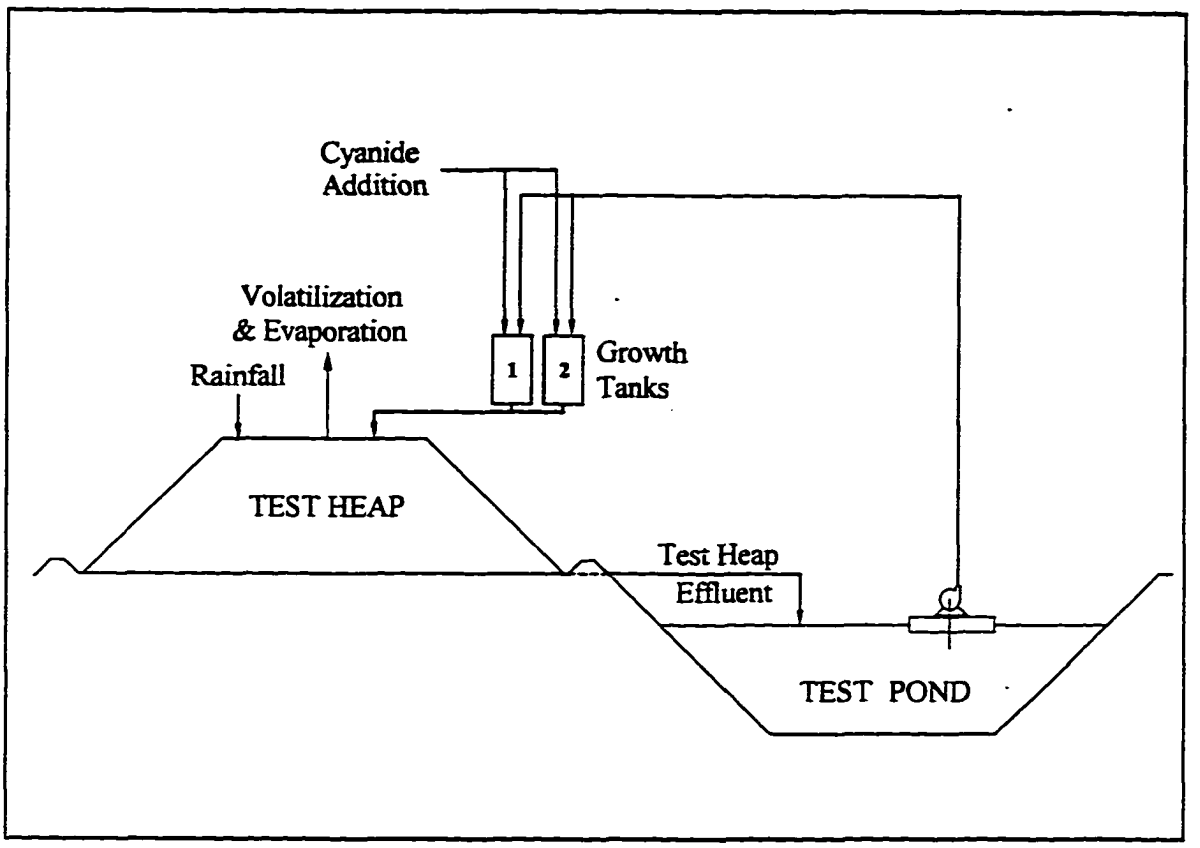


Figure 3.12 Test heap setup.

directly onto the liner and extended through the notch in the containment berm to aid in drainage from the test heap and leachate sampling.

A Caterpillar 225 backhoe was used to dig the test heap pond. After the hole was dug, two sheets of the Gundle 40 mil HDPE liner, welded together, were pulled into place. To fill the test pond, the end of one of the existing 5.1 cm PVC sprinkler lines from heap F was removed to fill the test pond with approximately 49,000 liters of water from the lower agglomeration pond.

At first, agglomerate was pushed over the edge of the top tier of F heap and

onto the test heap liner using a John Deere 450D tracked dozer. Because of the fine-grained nature, high clay content, and high cement content of the agglomerate it was concluded that the dozer would compact the test heap and create poor permeability if used to construct the entire test heap. Thus, the Caterpillar 225 backhoe was used to place the remaining agglomerate onto the test heap liner.

Two, 1,800 liter fiberglass tanks were placed on stands above the test heap and connected to a 5.1 cm PVC plumbing system as depicted in Figure 3.13. The tanks had previously been used as carbon stripping columns when the mine was actively leaching the ore. A one horsepower electric, centrifugal pump was used to draw water from the test pond into the tanks, and also to supply pressure to a sprinkler head on top of the test heap. Because there was no electrical power available at the test heap, a small gasoline generator was used to supply electricity to the components at the site.

Three, 6.4 cm diameter holes were drilled into the top of the test heap to a depth of approximately 2 m using a gas powered auger. Three, 2.5 m sections of 5.1 cm PVC pipe with 1.25 cm holes drilled through the pipe walls at 30 cm intervals were inserted into the holes in the top of the heap and connected to the piping system. This was to ensure that when the bacterial inoculum was applied to the heap, it would spread into the heap and speed in the colonization. A 5.1 cm ball valve was glued into each of the injection pipes to control the flow rate into the heap and to divert flow to the sprinkler head.

Because the agglomerate from heap F had been rinsed 3 seasons prior to the construction of the test heap, there was very little cyanide remaining. To test

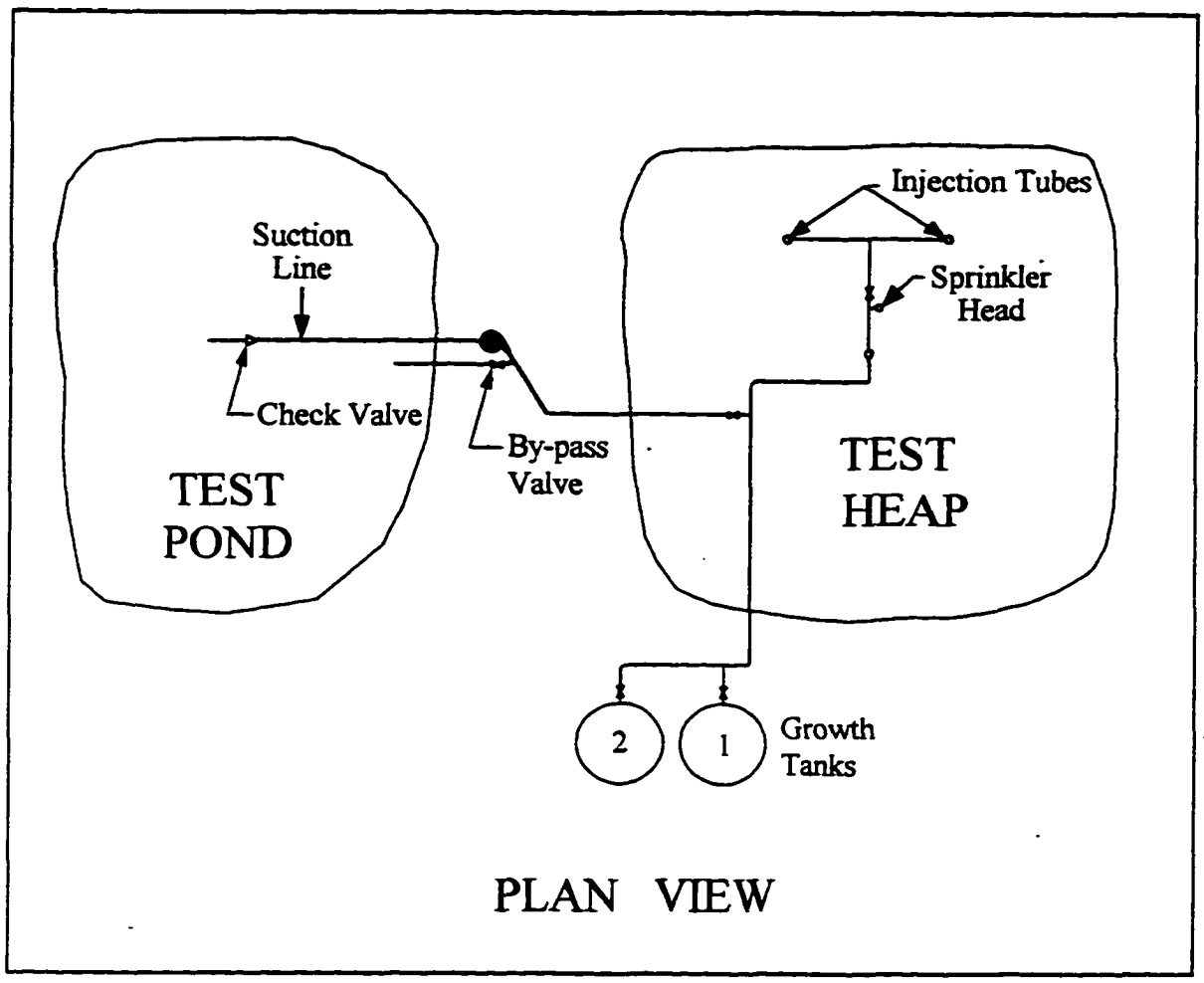


Figure 3.13 Piping system for the test heap.

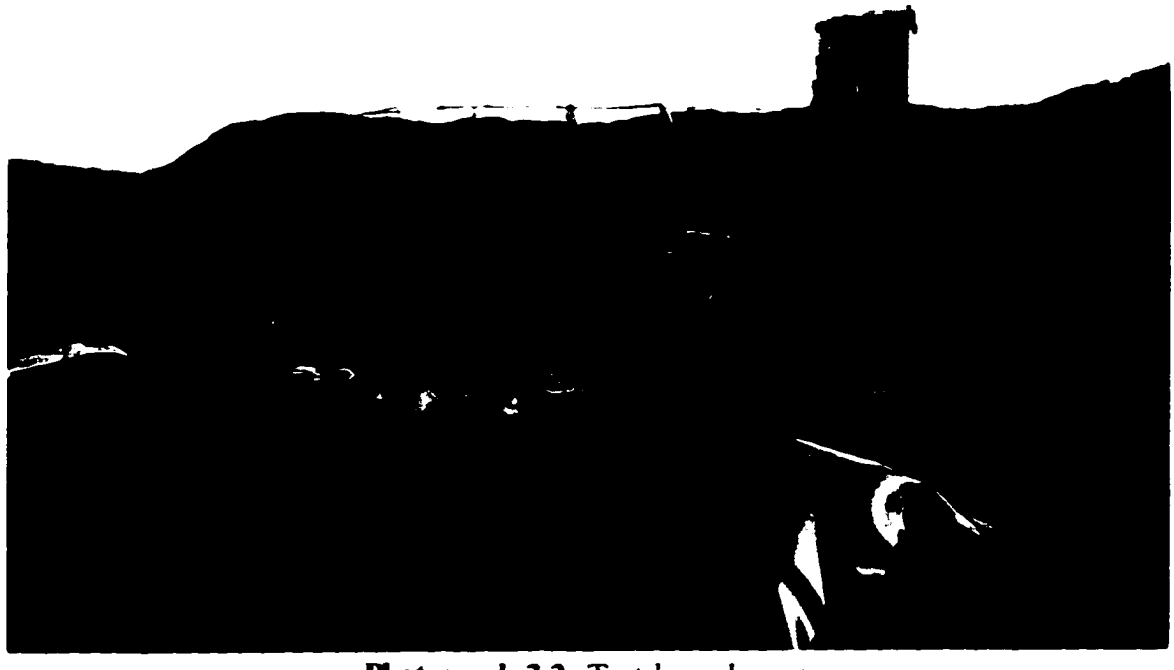
biological degradation, the target cyanide concentration in the agglomerate was chosen to be 100 mg/kg. To speed the addition of cyanide to the test heap, sodium cyanide briquettes were added to the tanks as they were filled with solution from the test pond, to make a solution of approximately 2,000 mg/l of CN^- in each tank. In addition to the sodium cyanide, sodium hydroxide was added to maintain a pH above ten in the solution.

Photograph 3.3 was taken from the southwest corner of the test site looking northeast, and depicts the layout of the test heap experiment. The pond is visible in the foreground of the photograph, the test heap in the center, and the growth tanks in the upper right. Photograph 3.4 shows the top of the test heap and the piping system.

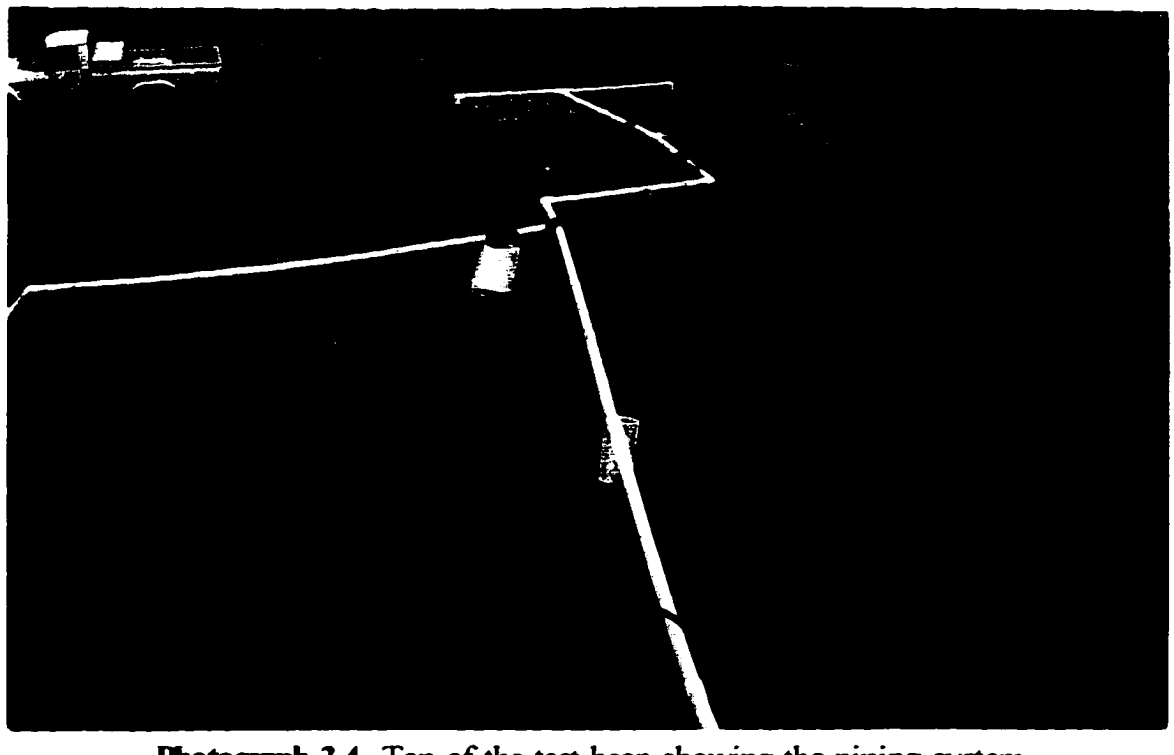
A 3/4 horsepower electric mixer with a 0.9 m stainless steel shaft and a 10.2 cm stainless steel propeller was used to mix the cyanide in each tank for approximately 20 minutes. While the solution in the tanks was mixing, the pump was providing solution from the test pond to the sprinkler head. Because of the high pressure created by the pump, a by-pass valve was opened and the flow to the sprinkler head was adjusted to get full sprinkler coverage of the test heap. The remainder of solution supplied by the pump was allowed to flow back into the test pond through the by-pass valve.

Once the tanks of solution were mixed, the pump was switched off and the valves at the tanks were opened and the solution was gravity fed to the sprinkler head. When only using gravity, the sprinkler head covered an area on top of the test heap of approximately 4.5 m in diameter. The two tanks, for a total of 3,600 liters, provided a supply of approximately 26 hours of solution to the sprinkler head. The test heap was completed on August 17, 1993 and solution from the test pond was applied to the heap.

The same tanks which were used to mix the cyanide solution were also used to grow the bacteria to inoculate the test heap. In order to grow 3,600 liters of bacteria, growth had to be started in the lab in the presence of cyanide. This was accomplished



Photograph 3.3 Test heap layout.



Photograph 3.4 Top of the test heap showing the piping system.

by preparing approximately 50 petri plates with PGY agar and 20 mg/l sodium cyanide. The plates were then inoculated with bacteria from the DMSO perms described in section 3.2.1. These plates were allowed to grow in the dark at 20° C for two weeks. Once there was sufficient growth on the PGY+20 mg/l CN^- plates, 20, two-liter flasks of PGY broth containing 20 mg/l CN^- were prepared and inoculated from the plates. These flasks were placed on shaker tables and grown at 20° C for about one week.

The bacterial populations grew quickly in the 20 flasks. They were transported to the test site on August 26, 1993. Both tanks were flushed thoroughly with clean water and the inoculum was added to tank one. In addition to the 20 liters of inoculum, tank one contained about 1,800 liters of full strength PGY broth and 20 mg/l CN^-. The bacteria were grown in tank one until the population reached a Klett density of about 60. On September 16, half of the solution in tank one was pumped into tank two, and clean water and nutrients were added to both tanks in order to bring the PGY broth back to full strength. On October 1, because of the onset of cold weather, the decision was made to go ahead and inoculate the heap with the bacteria from the tanks even though the cyanide concentration in the test heap was still below 10 mg/kg.

Beginning June 3, 1994, samples of the solution in the test pond, test heap effluent, and agglomerate were taken at periodic intervals. The test pond samples were taken from the by-pass line from the pump after it had ran for 10 minutes. The test heap effluent samples were taken directly from the slotted drain pipes extending

under the test heap. The agglomerate samples were constituted from four samples gathered at random places from a depth of six inches under the top of the heap. The samples were gathered in this manner in order to reduce the total cost of the cyanide analyses.

On September 3, 1994, another 75 liters of inoculum grown in the lab to a Klett density of 120, as described above, was transported to the test heap and tank one was inoculated. In order to grow the inoculum quicker, both tanks were wrapped with a 15 cm layer of fiberglass insulation and then wrapped with 10 mil black polyethylene (plastic). Heating elements were screwed into ports in the sides of the tanks, at about the half-way point in the tank's height. The generator was operated for about five hours per day, and heating elements maintained a temperature of about 25° C in the tanks during growth with the help of the insulation. The bacteria were grown in tank one until the population reached a Klett density of about 120, which took about nine days. Once the bacterial population reached this value, half the inoculum in tank one was pumped into tank two. Clean water, nutrients, and cyanide were added to both tanks in order to bring the PGY broth back to full strength. The bacteria grown for another 11 days and were finally applied to the heap on September 23, 1994.

3.6.2 Results from Test Heap Experiment

Table 3.7 indicates the dates and amounts of solution added to the test heap and the dates on which samples of the test heap agglomerate, test pond, and test heap

effluent were collected. Figure 3.14 is a plot of the total cyanide concentration verses the date, and depicts the rise in the cyanide concentration in the heap effluent with time.

The date June 24, 1994 is listed in bold because that is the date when solution began draining freely from the test heap into the test pond. This corresponds to about 28 days and 30,500 liters, near the calculated pore volume of the test heap.

The samples were sent to Northern Testing Labs (NTL) in Fairbanks and were assayed for WAD cyanide and for total cyanide using EPA Method 335.2 (Appendix B). Several of the sample results from the test heap reported by NTL indicated the WAD cyanide concentrations were well below the total cyanide results. This indicated that only a portion of the cyanide in the test heap was in the WAD form, so only the total cyanide results were used for analysis (Table 3.7).

The cyanide concentrations reported for the agglomerate samples are express as mg cyanide per kg dry solids. The reports also provide the percent solids for each agglomerate sample. Because there is no procedure to separate the cyanide concentration in the solids from the cyanide concentration in the pore liquid in a sample, NTL used Equation 3.1 to determine the total cyanide concentration on a dry weight basis

$$Total\ Cyanide = \frac{X}{\%\ Solids} \qquad (3.1)$$

where X is the cyanide concentration from the moist agglomerate samples. The results from each analysis are included in Appendix C.

Table 3.7 Test heap data.

Date	NaCN Added (pounds)	Volume of Solution (liters)	Concentration of Solution*	Volume of Rainfall (liters)	Measured Cyanide Concentrations (mg/liter)		
					Pond	Heap	Heap Effluent
03-Jun-94	0.0	0	0.0	0	0.12		
06-Jun-94	14.9	1790	2003.0	60			
07-Jun-94	0.0	0	0.0	360			
08-Jun-94	14.9	1790	2003.0	0			
10-Jun-94	29.8	3580	2003.0	780			
13-Jun-94	28.7	3580	1929.1	0			
14-Jun-94	0.0	0	0.0	360			
15-Jun-94	30.9	3580	2077.0	360			
16-Jun-94	0.0	0	0.0	720			
17-Jun-94	29.8	3580	2003.0	540			
18-Jun-94	0.0	0	0.0	1440			
19-Jun-94	0.0	0	0.0	960			
20-Jun-94	0.0	500	0.0	2280			
21-Jun-94	0.0	0	0.0	1620			
22-Jun-94	0.0	0	0.0	2100			
24-Jun-94	0.0	500	0.0	60			
25-Jun-94	0.0	0	0.0	1440			
26-Jun-94	0.0	0	0.0	60			
27-Jun-94	0.0	0	0.0	540			
01-Jul-94	7.0	3580	470.8	0	0.28		
05-Jul-94	0.0	0	0.0	420			
06-Jul-94	0.0	50	0.0	60			
07-Jul-94	0.0	3580	0.3	0			
11-Jul-94	0.0	3580	0.3	0			70.2
12-Jul-94	0.0	0	0.0	420			
15-Jul-94	0.0	3580	0.3	300			
16-Jul-94	0.0	0	0.0	360			
18-Jul-94	0.0	3580	5.8	480	5.76	185	227
20-Jul-94	29.8	3580	2008.8	300			
22-Jul-94	29.8	3580	2008.8	0			
25-Jul-94	29.8	3580	2014.6	0	11.6		201
26-Jul-94	0.0	0	0.0	2700			
27-Jul-94	0.0	0	0.0	1620			
29-Jul-94	29.8	3580	2014.6	0			

Table continued on following page

Table 3.7 continued.

Date	NaCN Added (pounds)	Volume of Solution (liters)	Concentration of Solution*	Volume of Rainfall (liters)	Measured Cyanide Concentrations (mg/liter)		
					Pond	Heap	Heap Effluent
02-Aug-94	29.8	3580	2017.8	60	14.8	166	228
03-Aug-94	27.8	3580	1883.4	0			
05-Aug-94	0.0	1000	0.0	0			
06-Aug-94	0.0	1000	0.0	0			
08-Aug-94	31.8	3580	2187.9	0	50.4	76	278
11-Aug-94	0.0	0	0.0	120			
12-Aug-94	0.0	4000	50.4	0			
15-Aug-94	12.3	3580	924.6	0	97.8	66	
17-Aug-94	0.0	0	0.0	600			
19-Aug-94	0.0	4000	97.8	0			
20-Aug-94	0.0	0	0.0	1080			
21-Aug-94	0.0	0	0.0	120			
22-Aug-94	0.0	0	0.0	60			
23-Aug-94	0.0	4000	52.7	0	52.7		350
24-Aug-94	0.0	0	0.0	1680			
25-Aug-94	0.0	0	0.0	2760			
29-Aug-94	0.0	0	0.0	0	91	61.25	417
01-Sep-94	0.0	0	0.0	240			
02-Sep-94	0.0	0	0.0	1080			
05-Sep-94	0.0	0	0.0	60			
07-Sep-94	0.0	0	0.0	60	156		630
08-Sep-94	0.0	0	0.0	960			
11-Sep-94	0.0	0	0.0	720			
12-Sep-94	0.0	0	0.0	0		27	
13-Sep-94	0.0	0	0.0	0			436
16-Sep-94	0.0	0	0.0	0	205		663.5
17-Sep-94	0.0	0	0.0	0	214		686
19-Sep-94	0.0	0	0.0	0	180		751
23-Sep-94	0.0	0	0.0	0	178		664
26-Sep-94	0.0	0	0.0	0	174		
30-Sep-94	0.0	0	0.0	0			659.5
06-Oct-94	0.0	0	0.0	0			675.5

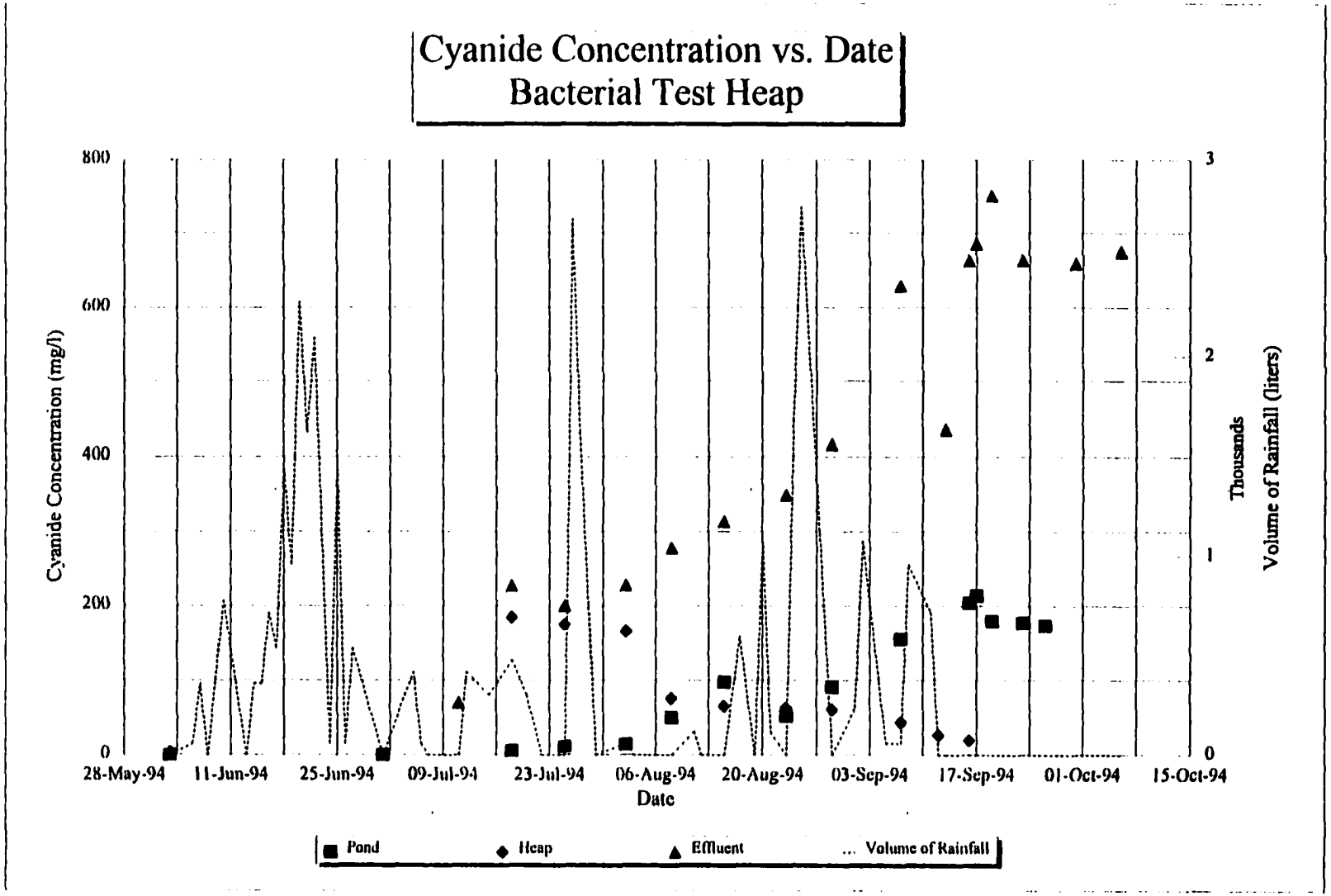


Figure 3.14 Cyanide concentration vs. date.

When the first cyanide assays were returned from NTL, it was noticed that the cyanide concentrations in the agglomerate and the heap effluent were not the same. This was expected, because of the way the cyanide solution was applied. Because the cyanide concentration of the solution applied to the top of the heap and effluent from the heap are known at the intervals listed in Table 3.7, it was assumed that the expression of the cyanide concentrations in the heap formed a straight line between the two points. Although this assumption was an oversimplification, it allowed the quantity of cyanide in the test heap to be determined, and made it possible to conduct a rough cyanide mass balance on the entire system. Equation 3.2 was used to calculate the cyanide mass balance from the cyanide concentrations listed in Table 3.7

$$m_{added} = m_{pond} + m_{heap} + m_{volatilized} + m_{degraded} \qquad (3.2)$$

where m is the mass of cyanide at the designated interval. For this equation to be used, values for the mass of cyanide volatilized and degraded were estimated to be 10% and zero, respectively. For the sampling dates, the cyanide mass in the test heap system was calculated using Equation 3.3

$$C_{heap} = \frac{\left(m_{added} - C_{pond}V_{pond} \right) 0.9}{m_{agglomerate}} \qquad (3.3)$$

where C_{heap} = cyanide concentration in the test heap in mg/dry kg, C_{pond} = cyanide concentration in the test pond in mg/l, V_{pond} = volume of solution in the test pond in liters, and $m_{agglomerate}$ = total mass of the test heap agglomerate in kg. Figure 3.15 was created using Equation 3.3 and depicts the rise in cyanide mass in the test system over

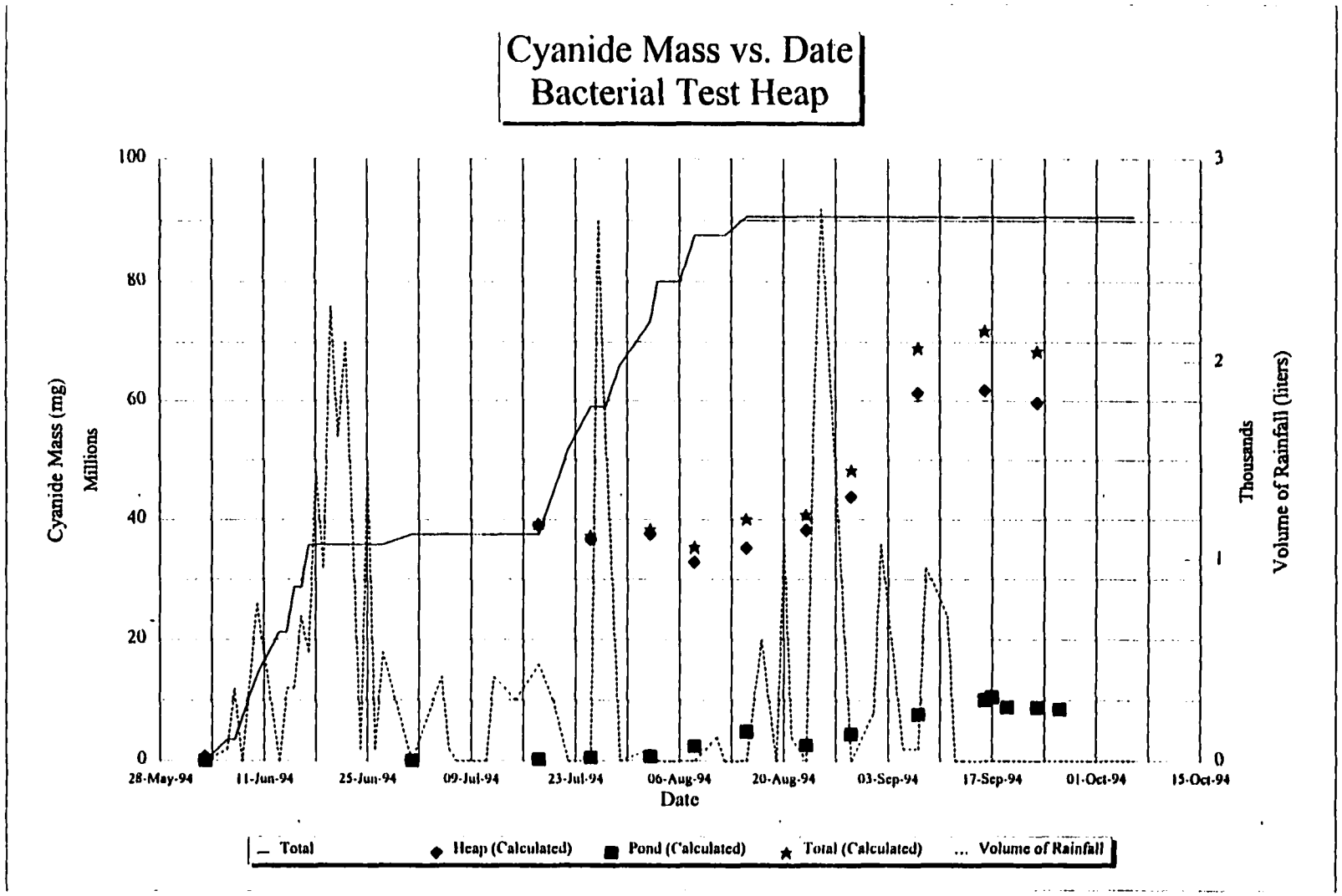


Figure 3.15 Cyanide mass verses date.

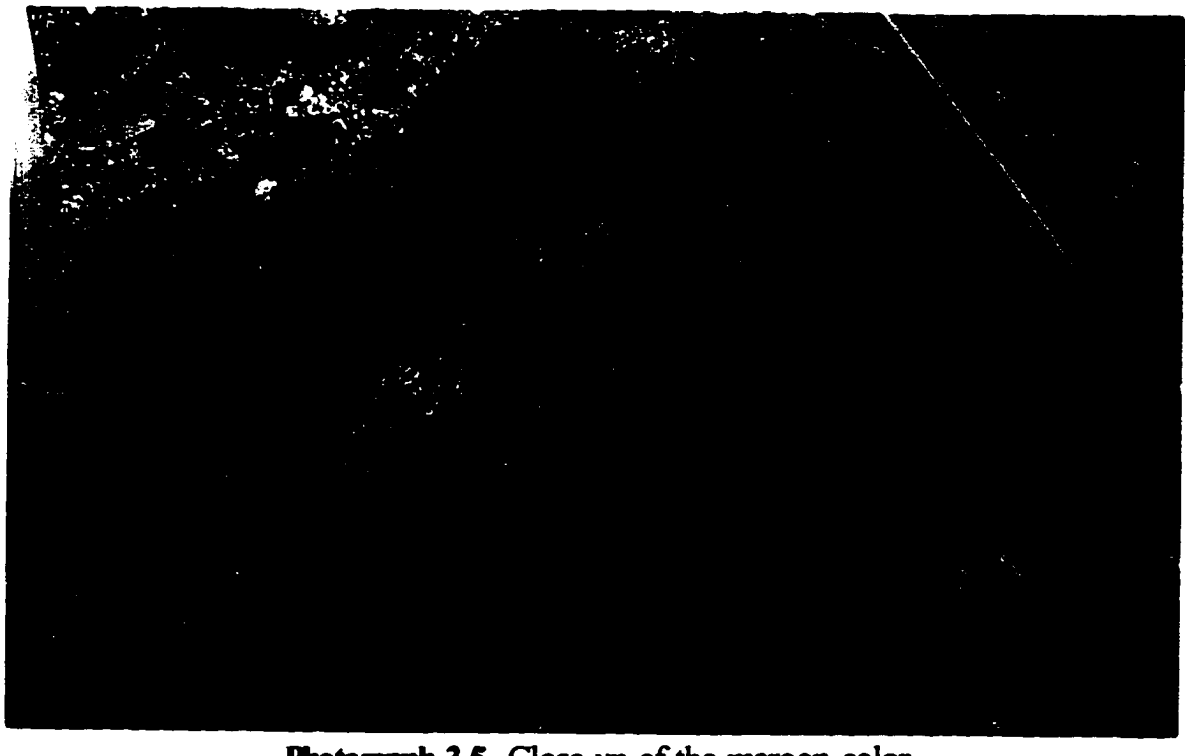
time.

There are two interesting items to note from Figure 3.15. First, the curves for the cyanide mass in the effluent and the pond very closely follow the cumulative cyanide mass curve with a time delay of about 28 days, which is the time required to replace one pore volume of solution at the sprinkling rate used during testing. It can also be seen from Figure 3.15 that the cyanide mass in the heap started a downward trend at the end of the sampling period. If the test had been allowed to continue, positive proof of bacterial degradation would have been recorded from this test.

Starting around July 1, during the same period cyanide solution was being applied to the test heap surface, it was noticed that the surface of the test heap had taken on a maroon color and was giving off odors which were characteristic of *Pseudomonas*. Photograph 3.4 was taken July 18, 1994, and shows the top of the test heap and the areal extent of the maroon color. Photograph 3.5 is a close-up of an area where the maroon color was very strong. The bacterial growth was most likely due to the survival of a portion of the bacterial population inoculated into the test heap on October 1, 1993, since it is unlikely that any natively occurring microorganisms could survive the sprinkling of the solution, which contained 2,000 mg/l cyanide. This bacterial growth could be the reason the cyanide concentrations in the heap agglomerate did not rise with the addition of cyanide.

Photograph 3.6 shows the notch in the containment berm, and also the perforated plastic drain tiles at the bottom of the heap, where the samples of the heap effluent were taken. The maroon color is also visible in this photograph, with the

strongest color near the heap effluent. Again, it is doubtful that any natively occurring microorganisms could survive in this area due to the cyanide content of the test heap effluent, which was 200 mg/l at the time the picture was taken.



Photograph 3.5 Close-up of the maroon color.

Photograph 3.6 The notch in the containment berm and perforated plastic drain tiles.

CHAPTER FOUR

4.0 ECONOMIC ANALYSIS

Even after the successes described in the previous chapters, there are few data published about the specific cost savings that could result from biological detoxification, either in situ or ex situ. Several authors have compared the costs associated with various detoxification methods (Anonymous, 1995; Drozd, 1992; Mosher and Figueroa, 1996), but have provided general or little data on the specific cost savings of using biological cyanide detoxification compared to the various chemical detoxification methods. This chapter addresses this issue and compares seven different cyanide detoxification methods utilizing data and cost estimates for a hypothetical heap of one million tons in the Fairbanks area.

4.1 Background

Several factors affect the use of any process in the mining industry. Some of the most important that affect the choice for the cyanide detoxification process for most mines are:

Availability
Familiarity
Ease of use
Efficiency
Reliability
Cost

Each of these factors will be examined in detail below.

4.1.1 Availability

In the Fairbanks area, as with most of the U.S., goods can reach their destination through a variety of transportation modes. Therefore, all the necessary supplies for any of the cyanide detoxification methods described in Chapter one could be available. However, remote mine sites in Alaska usually only have two transportation methods available to get supplies to the mine site; air and temporary winter roads. Shipping supplies by air can be very expensive, and in some cases could far exceed the cost of the goods being transported. Winter roads offer an alternative to expensive air shipment, but the cost for shipping goods by winter roads is still more expensive than traditional paved roads. One mine which has utilized both air shipment and winter roads is the Nixon Fork Mine, just south of McGrath, Alaska. For remote mines such as the Nixon Fork Mine, transportation of the reagents adds to the cost of any cyanide detoxification method and could make any process which requires large quantities of reagents cost prohibitive.

4.1.2 Familiarity

This is a problem for new processes being placed in the market place, and biological cyanide detoxification is no exception. Most miners, metallurgists, and engineers are hesitant to utilize processes with which they are not familiar. This is especially the case when the cyanide detoxification process affects the status of the

mine permits.

Some of the techniques used by companies to expose a new process to the industry are displays at trade shows, publication of news releases, publication of articles in technical and trade journals, cooperative research with universities and research organizations, and active sales to mining companies either in production or at the planning stage. Biological cyanide detoxification has had increasing exposure, and been used in some commercial applications (Thompson, et al., 1994).

4.1.3 Ease of Use

The ease of use of a cyanide detoxification process can greatly affect its cost. The more technical the process, the greater the need for highly trained personnel and the higher the number of visits to the mine site by the inventors of the process, or those skilled in its application. A process which is highly technical would probably require more technology to control, which would also drive up its cost.

4.1.4 Efficiency

The overall efficiency of cyanide detoxification processes must be examined before a specific process is chosen. Some of the parameters that affect the overall efficiency include the chemistry of the wastewater, final cyanide level, rate of consumption of reagents, and the amounts of by-products or wastes from the process. In Alaska the treatment levels are usually specific to a given mine permit; generally, the wastewater must be below 0.2 mg/l WAD cyanide, and the total cyanide in the

solids must be below 5 mg/kg dry solids.

4.1.5 Reliability

The agencies regulating the mining industry look at the entire mine and reclamation plan before issuing permits to the mining company. In response to the increasing pressure from environmental groups, these regulatory agencies are now scrutinizing mine plans very closely, especially when the plans involve the use of cyanide. Therefore, the regulatory agencies are hesitant to approve mine plans containing cyanide detoxification processes that are experimental or have not been proven to be as reliable as competitive processes. The chemical cyanide detoxification methods employed at major mines throughout the U.S. today have been proven to be highly reliable.

4.1.6 Cost

The overall cost is usually the deciding factor when choosing a cyanide detoxification process. The cost of the detoxification process must be included in the overall financial analysis of any project and increases in that cost would decrease the overall profitability of the project, making the project less attractive. Since the cost is usually the deciding factor, it will be treated in depth in the following sections.

4.2 Cost Comparison

In the remainder of this chapter, the costs associated with seven cyanide

detoxification processes will be compared. Several assumptions must be made in order to complete the comparisons. These assumptions are stated following sections.

4.2.1 Assumptions

To compare the detoxification costs of the different processes, a hypothetical spent heap of one million tons of ore, at a density of 100 pounds per cubic foot, was used for all calculations. The hypothetical heap was chosen to be square and 25 feet high and roughly 900 feet on a side. The specific yield of the hypothetical heap was chosen to be 0.25, and the pore volume calculated as approximately 37,400,000 gallons. The maintenance costs for each method were estimated at 5% of the capital costs.

It was assumed that there was existing electrical service at the mine site and electricity was purchased from Golden Valley Electric Association at the rate of $0.13032 per kilowatt-hour.

To reduce the capital cost expenditures, it was assumed the pumping system used to pump the pregnant solution from the heap was in-place and available for use in the cyanide detoxification circuit. To further reduce the capital costs, it was assumed the existing barren or pregnant pond could be used, rather than constructing a new one for heap detoxification.

In Alaska, the average detoxification season at a mine is generally governed by the weather, with the ponds being ice-free from around May 1st to October 1st, or about 150 days a year in total. In the calculations for the hypothetical heap, an

assumed rinse solution flow rate of 1,000 gallons per minute (gpm) for eight hours a day resulted in pumping one heap pore volume (37,400,000 gallons) every 78 days, which is approximately half a rinsing season in Alaska.

To adequately circulate the rinse solution and promote evaporation, it was assumed that a sprinkler grid would need to be constructed.

The rinse solution pumping rate was selected as 1,000 gpm, but in a real situation would depend on the material properties of the heap. Given these flow rates, the cyanide concentration in the hypothetical heap after each summer of rinsing could be determined. The starting cyanide concentration in the leachate was chosen as 250 mg/l, which is inserted into Equation 2.2, yielding Equation 4.1

$$Y = 250 * e^{\left(-0.111 * X^{2.97}\right)} \qquad (4.1)$$

where, Y is the cyanide concentration in the hypothetical heap after X pore volumes of rinsate applied to the heap. Using this equation, the cyanide concentration in the hypothetical heap effluent was calculated for five pore volumes of rinsate and the results are listed in Table 4.1.

As described in section 2.2.3, the results from Thompson's work showed that in situ biological cyanide detoxification required less time than competitive chemical methods. Using this premise, the B constant, -0.111, in Equation 4.1 was modified to provide curves which more closely matched the curves obtained by Thompson (Figure 2.10). The B constants were changed as follows: to -0.25 for in situ biological, to -0.2 for H_2O_2, and to -0.15 for $Ca(OCl)_2$. The resultant curves were used in the cost

comparison analyses, and are listed in Table 4.1 and plotted in Figure 4.1. For ex situ methods, the time required to detoxify the hypothetical heap was assumed to be the same as rinsing for all four methods.

Table 4.1 Cyanide concentrations (mg/l) for the hypothetical heap at various pore volumes of rinsate.

Number of Pore Volumes	In situ Biological	In situ Peroxide	In situ Chlorination	Ex situ Methods
0	250	250	250	250
1	194.7	204.7	215.2	224
2	33.8	50.5	75.3	105
3	0.29	1.13	4.36	13.8
4	2.8×10^{-5}	6.9×10^{-4}	0.017	0.27
5	-	-	-	4.5×10^{-4}

Using a final WAD cyanide concentration of 0.2 mg/l (see Section 4.1.4), the hypothetical heap did not quite reach the final goal of 0.2 mg/l after the fourth pore volume, and required a fifth pore volume of rinsing, as shown in Table 4.1. All the in situ methods, however, reached 0.2 mg/l after the fourth pore volume.

4.2.2 Costs

To compare the costs of the different cyanide detoxification methods, a list of the required materials and their costs was needed. Table 4.2 is a compiled list the

materials required for each of the detoxification processes. This list may not reflect the materials which are required at every mine site, but instead is a list of the pertinent materials for detoxification of the hypothetical heap.

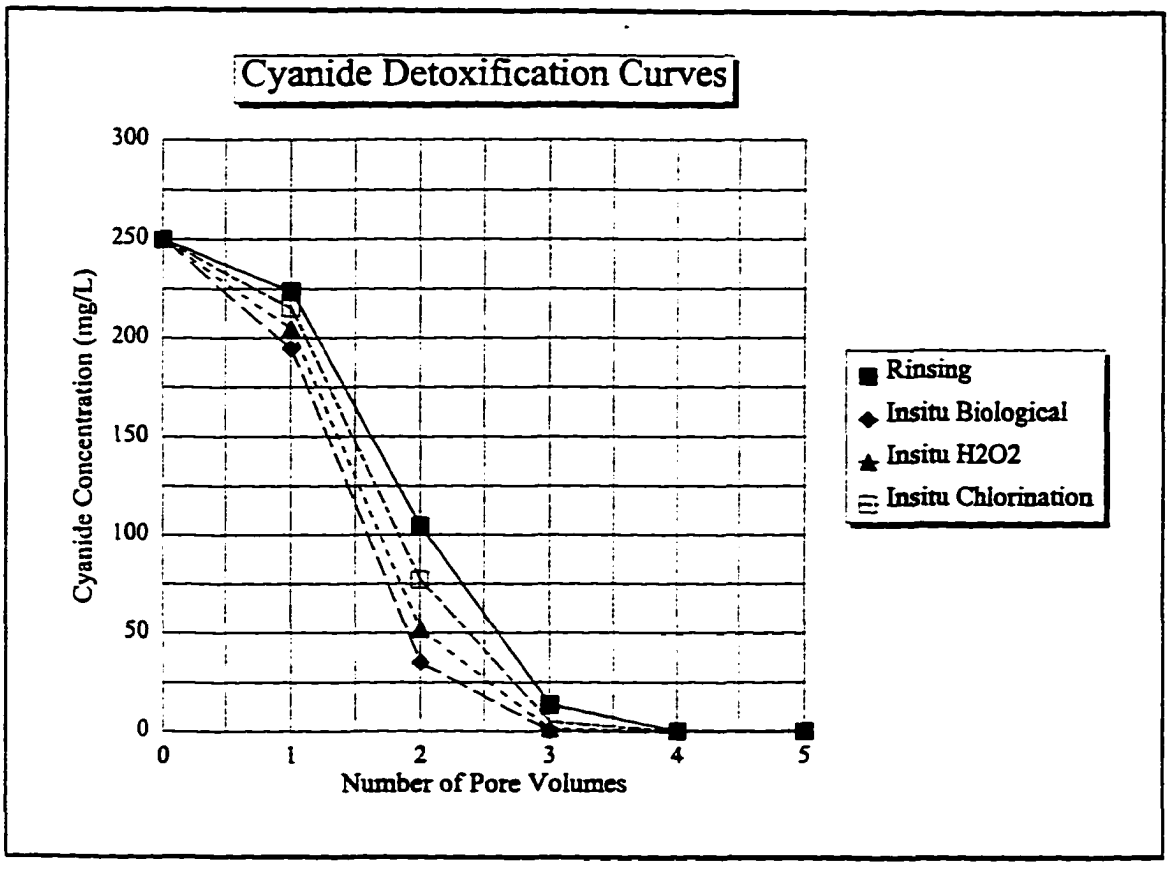


Figure 4.1 Cyanide detoxification curves used for cost comparison.

One of the major costs of heap detoxification is personnel. Table 4.3 shows the number, classification, hourly rate, average number of hours worked per week, and number of weeks worked per year for each employee.

Table 4.2 List of capital costs for each method.

Capital Costs:	INSITU TECHNIQUES			EXSITU TECHNIQUES			
	Biological	Peroxide	Chlorination	Biological	INCO	Peroxide	Chlorination
Heap sprinkler system	$74,275	$74,275	$74,275	$74,275	$74,275	$74,275	$74,275
Aeration system	$0	$0	$0	$0	$1,176	$0	$0
Mixing Systems	$591	$591	$591	$591	$591	$591	$591
Biological Treatment Plant	$0	$0	$0	$317,950	$0	$0	$0
Microbiology Lab	$14,500	$0	$0	$14,500	$0	$0	$0
Totals	$89,366	$74,866	$74,866	$407,316	$76,042	$74,866	$74,866

			INSITU TECHNIQUES			EXSITU TECHNIQUES			
	Quantity	Unit Price	Biological	Peroxide	Chlorination	Biological	INCO	Peroxide	Chlorination
Heap sprinkler system									
2" PVC slip-joint pipe (sched 40)	32500	$1.12	$36,309	$36,309	$36,309	$36,309	$36,309	$36,309	$36,309
4" PVC slip-joint pipe (sched 40)	2000	$3.31	$6,612	$6,612	$6,612	$6,612	$6,612	$6,612	$6,612
6" PVC slip-joint pipe (sched 40)	1000	$5.81	$5,813	$5,813	$5,813	$5,813	$5,813	$5,813	$5,813
6" slip-joint check valve	2	$150.00	$300	$300	$300	$300	$300	$300	$300
6" slip-joint ball valve	5	$250.00	$1,250	$1,250	$1,250	$1,250	$1,250	$1,250	$1,250
4"x4"x2" PVC tees	150	$13.55	$2,033	$2,033	$2,033	$2,033	$2,033	$2,033	$2,033
2" PVC end cap	150	$0.76	$114	$114	$114	$114	$114	$114	$114
Quart of PVC primer	50	$6.98	$349	$349	$349	$349	$349	$349	$349
Quart of PVC glue	50	$7.42	$371	$371	$371	$371	$371	$371	$371
#8 Senninger wobbler head	2500	$8.25	$20,625	$20,625	$20,625	$20,625	$20,625	$20,625	$20,625
Miscellaneous pieces	1	$500.00	$500	$500	$500	$500	$500	$500	$500
Total			$74,275	$74,275	$74,275	$74,275	$74,275	$74,275	$74,275
Aeration system									
2" PVC slip-joint pipe (sched 40)	150	$1.12	$0	$0	$0	$0	$168	$0	$0
4" PVC slip-joint pipe (sched 40)	150	$3.31	$0	$0	$0	$0	$496	$0	$0
#8 Senninger wobbler head	50	$8.25	$0	$0	$0	$0	$413	$0	$0
Miscellaneous pieces	1	$100.00	$0	$0	$0	$0	$100	$0	$0
Total			$0	$0	$0	$0	$1,176	$0	$0
Mixing Systems									
2" slip-joint ball valves	5	$65.97	$330	$330	$330	$330	$330	$330	$330
2" slip-joint check valves	2	$80.64	$161	$161	$161	$161	$161	$161	$161
Miscellaneous pieces	1	$100.00	$100	$100	$100	$100	$100	$100	$100
Total			$591	$591	$591	$591	$591	$591	$591
Biological Treatment Plant									
Rotating Biological Contactor	1	$316,950	$0	$0	$0	$316,950	$0	$0	$0
Miscellaneous pieces	1	$1,000	$0	$0	$0	$1,000	$0	$0	$0
Total			$0	$0	$0	$317,950	$0	$0	$0
Microbiology Lab									
Autoclave	1	$5,000	$5,000	$0	$0	$5,000	$0	$0	$0
Clean bench	1	$3,500	$3,500	$0	$0	$3,500	$0	$0	$0
Shakers	2	$1,500	$3,000	$0	$0	$3,000	$0	$0	$0
Chemicals	1	$1,000	$1,000	$0	$0	$1,000	$0	$0	$0
Glassware	1	$1,000	$1,000	$0	$0	$1,000	$0	$0	$0
Miscellaneous supplies	1	$1,000	$1,000	$0	$0	$1,000	$0	$0	$0
Total			$14,500	$0	$0	$14,500	$0	$0	$0

Table 4.3 List of labor costs for each method.

				INSITU TECHNIQUES			EXSITU TECHNIQUES			
				Biological	Peroxide	Chlorination	Biological	INCO	Peroxide	Chlorination
Yearly cost of Personnel:				$123,464	$96,464	$96,464	$123,464	$96,464	$96,464	$96,464
Classification:	Mean Hourly Rate*	Average Number of Hours per week	Number of Weeks Worked per Year	Cost per Year						
Professional:										
Mining engineer	$33.78	10	52	$17,566	$17,566	$17,566	$17,566	$17,566	$17,566	$17,566
Microbiologist	$30.00	30	30	$27,000			$27,000			
Chemist	$22.68	20	30	$13,608	$13,608	$13,608	$13,608	$13,608	$13,608	$13,608
Non-Professional:										
Operating engineer	$31.55	10	30	$9,464	$9,464	$9,464	$9,464	$9,464	$9,464	$9,464
Mine laborer	$18.02	60	30	$32,427	$32,427	$32,427	$32,427	$32,427	$32,427	$32,427
Support staff	$22.50	20	52	$23,400	$23,400	$23,400	$23,400	$23,400	$23,400	$23,400

* Includes 50% benefits

Source: Alaska Department of Labor, 1995

4.2.2.1 In situ Biological Detoxification

Phosphoric Acid

For the hypothetical heap, phosphoric acid (H_3PO_4) was added to the water at a concentration of 25 mg/l for use as a nutrient source for the bacteria. In the research reported by the USBM (Lien, et al., 1990) it was found that phosphoric acid addition above 25 mg/l had little or no further effect on the cyanide oxidation rate. The phosphoric acid acted in two ways: First, it reduced the pH of the water, and second, it supplied phosphate, which has been used as a biological nutrient in many industrial settings (Whitlock and Mudder, 1986).

Growth Medium

The nutrients used for cost estimation to grow the bacteria in the lab and in the growth tanks was a standard broth composed of peptone, glycerol and yeast extract (PGY), at full (100%), 50%, and 5% strength. The specific recipe for PGY can be found in Appendix H. There were two purposes for using a 5% PGY broth for growth of the bacteria. First, the low concentration of medium would tend to keep the size of the bacteria small, allowing for better dispersion of the bacteria throughout the heap. The second reason for using the 5% PGY was simple economics. If the bacteria will grow to the same population whether using full-strength or 5% PGY broth, with the only difference being the time required for the bacteria to reach that final population, there was substantial cost savings associated with using 5% PGY broth. The same PGY broth was used during the field testing, described in section 3.5. Laboratory-

grade reagents were purchased from VWR Scientific, and had a cost of $1.55 and $0.0775 per gallon of broth when mixed at 100% and 5% strength, respectively.

Biological Growth System

From published research (Thompson, et al., 1995a; Thompson, 1990; Thompson and Gerteis, 1990) it was estimated that approximately 80,000 gallons of 5% PGY broth were required to inoculate the hypothetical heap during the first year. The surface area of the heap was calculated to be approximately 800,000 square feet. The application rate of inoculum equates to approximately 0.1 gallons per square foot of heap surface. At a Klett reading of 90, there are approximately 1.5×10^{12} bacteria per gallon of inoculum, or a population applied to the surface of the heap of roughly 1.5×10^{11} bacteria per square foot.

Because tanks are generally used on the mine site for various processes, it was assumed that these spare tanks could be used to grow the inoculum. An alternative to growing the bacteria in tanks would be to construct a temporary pond in which the bacteria could be grown. If a five foot deep pond were constructed it would be roughly 50 feet on a side, in order to satisfy the 80,000 gallon requirement, and would require approximately 4,000 square feet of synthetic liner. The current price of 40 mil synthetic liner is approximately $0.50 per square foot, thus a total cost of $2,000 would be needed for the liner. Including labor for the earth work and installation, the total cost of the lined pond would be in the range of $3,500, which is considerably cheaper than buying new tanks, if none were available at the mine site.

Microbiology Lab

In order to complete a successful biodetoxification, a mine needs to have trained personnel and the laboratory facilities to grow and monitor a population of microorganisms. It was estimated that $14,500 of equipment and chemicals would be required to adequately stock the microbiology lab. Smaller mining companies could probably rely on a nearby university or third party to perform a major portion of the biological work, resulting in a cost savings.

4.2.2.2 In situ Peroxide Detoxification

Hydrogen Peroxide

The hydrogen peroxide was priced at Great Western Chemical, a local chemical supply house, in 55 gallon drums at 70% H_2O_2 and included a discount for large quantities. As stated in section 1.3.2.3, for industrial processes, the typical H_2O_2 addition rate is two to five times the cyanide concentration. Smith and Mudder (1991) suggest using a ratio of 7 lb H_2O_2 per lb cyanide to complete any side and dead-end reactions. Therefore, this was the ratio selected for the hypothetical heap.

4.2.2.3 In situ Chlorination

Calcium Hypochlorite

The calcium hypochlorite [$Ca(OCl)_2$] was also priced at Great Western in 50 pound buckets and included a discount for large quantities. The stoichiometric chlorine addition rate is 2.75 times the cyanide concentration. This equates to a

$Ca(OCl)_2$ addition rate of 5.5 times the cyanide concentration. In practice, the chlorine addition rate can increase by a factor of three or four. Therefore, the $Ca(OCl)_2$ addition rate for the hypothetical heap was selected as eight.

Sodium Hydroxide

Sodium hydroxide or lime is typically added to the wastewater along with the calcium hypochlorite to control the pH of the solution, which should be near 10.5 for the reactions to occur as described in section 1.3.2.1. The addition rate is dependent on the pH of the heap effluent, but is typically in the range of four to eight times the amount of cyanide destroyed. For the hypothetical heap, the sodium hydroxide/cyanide destroyed ratio was selected to be six.

4.2.2.4 Ex situ Biological Detoxification

Biological Treatment Plant

The type of biological treatment plant chosen was a rotating biological contactor (RBC), similar to the wastewater treatment plant in operation at the Homestake mine described in section 2.2.1. It was difficult to find recent references for estimating the capital cost of an RBC plant, however, R. L. Antonie (1976) provided good information on the design, construction, operation, and maintenance of RBC plants. The total installation costs were provided in a series of curves for different plant configurations. The total installation costs included RBC equipment, concrete tankage at $\$200/yd^3$, fiberglass enclosures, freight within the contiguous U.S.,

and installation costs for cranes, millwrights, electricians, etc. Configurations three and four most closely matched the type of system needed for the hypothetical heap, therefore that configuration was used for estimating the cost. The key elements for configurations three and four were 25,000 ft^2 of media per shaft, four-stage operation with four shafts, flat bottom tanks, and flow perpendicular to the shaft. The hydraulic loading was chosen as 2.5 gallons per day (gpd) per ft^2 of media surface.

The tables provided for estimating total installation costs were expressed in dollars per gpd versus the hydraulic loading. Using the sprinkling rates provided above, the flow rate from the hypothetical heap was calculated as 480,000 gpd. To add a small margin for safety, the flow rate was increased to 500,000 gpd. Using the hydraulic loading of 2.5 gpd/ft^2, and the flow rate of 500,000 gpd, the required media surface area was calculated as 200,000 ft^2. This would tend to suggest that the flow would have to be separated into two parallel streams for adequate treatment as depicted in Figure 4.3. Because the plant chosen was small, with short shafts, the value obtained from the chart was $0.30 per gpd, or $150,000 installed.

Since the reference was based on 1976 dollars, the $150,000 total installation cost was substantially lower than the 1996 cost. To correlate the 1976 cost to 1996 dollars, the Engineering News Record Building Cost Index (BCI) was used. The 1976 total installation cost was divided by the December 1976 BCI average for the twenty major cities and then multiplied by the March 1996 BCI average, which gave a total installation cost in 1996 dollars of $316,950.

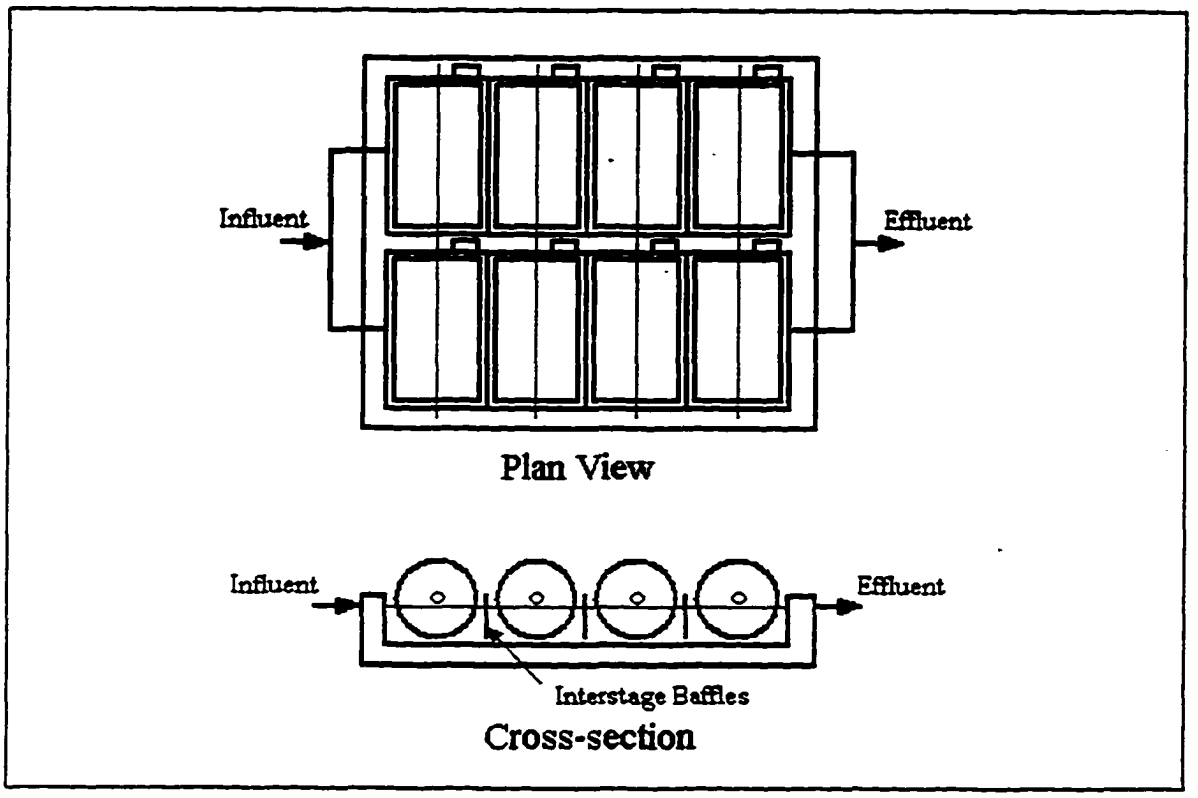


Figure 4.3 Rotating biological contactor configuration #3 (from Antonie, 1976).

Growth Medium

To inoculate the RBCs with bacteria, it was anticipated that the RBC tanks would be filled with heap effluent and a dextrose would be added at the rate of 0.23 g/L. The bacteria would use this as a carbon source and the cyanide as a nitrogen source. The RBCs would be placed into operation once sufficient growth was established on the disk surfaces and cyanide degradation was confirmed. The cost of the dextrose was calculated as $0.131 per gallon using costs obtained from VWR Scientific, with approximately 100,000 gallons required for adequate inoculation.

Phosphoric Acid

As with the in situ biological process, phosphoric acid (H_3PO_4) was added to the heap effluent at a concentration of 25 mg/l for use as a nutrient source for the bacteria.

Microbiology Lab

Similar to the in situ biological process, a microbiology lab would also be needed for ex situ biological process with a capital cost of $14,500.

4.2.2.5 INCO air-SO_2 System

Aeration System

The INCO process requires aeration of the rinse solution to complete the cyanide detoxification. For the hypothetical heap, this was accomplished using a floating sprinkler grid. The cost of the floating sprinkler grid was calculated at $557, not including the pump. The floating sprinkler grid was driven by a centrifugal pump placed on a float in the pond.

Sodium metabisulfite

For cost estimation, sodium metabisulfite was added to a mixing chamber at the detoxification pond at a rate of eight times the cyanide concentration in the inflow water. This may be slightly excessive for some applications, but these were the ratios used at the Ryan Lode mine during cyanide detoxification.

The sodium metabisulfite was priced at Great Western Chemical, a local chemical supply house, in 50 pound bags and included a discount for large quantities. Personal conversation with INCO personnel indicated that INCO is now using ammonium bisulfite instead of the sodium metabisulfite, and that ammonium bisulfite is about one-fifth the price of sodium metabisulfite. However, Great Western Chemical did not have a price available for ammonium bisulfite, so it was decided that sodium metabisulfite would be used in this analysis. Another reason for using sodium metabisulfite instead of ammonium bisulfite was that the ratio of sodium metabisulfite to cyanide was known for the Ryan Lode Mine at the time of the cost estimation.

Sodium hydroxide

Sodium hydroxide is typically added to the mixing chamber at a rate of eight times the cyanide concentration in the inflow water along with the sodium metabisulfite to control the pH of the water going into the pond. From personal experience, the sodium hydroxide addition rate is usually in the range of 75-150% the sodium metabisulfite addition rate. This equates to about six to twelve times the cyanide concentration in the inflow water and depends on the pH of the pond.

$$CN^- + SO_2 + O_2 + Cu + H_2O = CNO^- + Cu + H_2SO_4 \qquad (4.2)$$

Equation 1.20, reproduced here, indicates that sulfuric acid (H_2SO_4) is generated during the reaction of SO_2 with cyanide, which lowers the pH of the water and creates the necessity for sodium hydroxide addition.

The sodium hydroxide was also priced at Great Western Chemical in 50 pound bags and again included a discount for large quantities.

4.2.2.5 Ex situ Peroxide Detoxification

The same data used for in situ peroxide detoxification were used for the ex situ peroxide detoxification process.

4.2.2.6 Ex situ Chlorination

The same data used for in situ chlorination were used for the ex situ chlorination process.

4.2.3 Cost Comparison Results

The PC software package "Software for Economic Evaluation" (SEE) was used to compare the detoxification costs associated with the different processes. SEE automatically discounts all the costs to a common date, expressed as the present worth cost (PWC), allowing for comparison of alternatives with different periods of life on an equal basis. SEE also allows for several variables, such as taxes, inflation, and the internal rate (IRR) of return to be set at the user's option. For the cost comparison calculations, the inflation and IRR were set to zero and the cost comparison was made before taxes.

All seven detoxification processes were analyzed first in a spreadsheet, and then with SEE, with the results listed in Table 4.3. A detailed output from the

spreadsheet is provided in Appendix F and the SEE output is provided in Appendix G.

Table 4.3 Results from the cost comparison.

	Detoxification Cost
In situ Biological	$413,105
Ex situ Biological	$919,311
In situ Peroxide	$1,049,362
In situ Chlorination	$1,151,389
INCO Air-SO_2	$1,294,467
Ex situ Peroxide	$1,325,246
Ex situ Chlorination	$1,354,434

For the conditions analyzed, it can be seen that in situ biodetoxification was two to three times less expensive than other competitive detoxification methods. The two biodetoxification methods required higher capital and labor costs. Those costs, however, were offset by the low operating costs.

4.3 Sensitivity Analysis

In addition to the most likely cost comparison provided above, a sensitivity analysis using SEE was also conducted on the seven different detoxification processes to determine to which variables the costs were most sensitive. To obtain the sensitivity of each variable on the total detoxification costs, one input variable was changed while the remaining variables were held constant, and the total detoxification

cost was recalculated. The recalculated total detoxification cost was plotted against the percentage of the input variable. For this analysis, the input variables were changed incrementally from 25% to 200% of the most likely cost. The variables analyzed and the results of the sensitivity analysis for each detoxification process are listed in Tables 4.4 to 4.10 and plotted in Figures 4.3 to 4.9. The data from the sensitivity analysis are provided in a decimal fraction of the total costs for each input variable. The data formed a straight line except for the rinsing rate. A power series line displayed the highest correlation coefficient, so the data for the rinsing rate was analyzed, and the results are provided as an equation for each process, where x is the rinsing rate in gpm.

Table 4.4 Sensitivity analysis for the In situ Biological Process.

Input Variable	Sensitivity
Rinsing Rate	$1.093 \times ^{-0.625}$
Yearly Labor Cost	0.598
Capital Costs	0.216
Phosphate Concentration	0.072
Phosphate Cost	0.072
Electricity Cost	0.059
Growth Medium Strength	0.034
Growth Medium Cost	0.034
Inoculum Volume	0.034
Yearly Maintenance Cost	0.022
Starting Cyanide Concentration	0.019

Table 4.5 Sensitivity analysis for the In situ Peroxide Process.

Input Variable	Sensitivity
Oxidant/Cyanide Ratio	0.715
Oxidant Cost	0.715
Starting Cyanide Concentration	0.715
Rinsing Rate	$1.000 \times ^{-0.25}$
Yearly Labor Cost	0.184
Capital Costs	0.071
Electricity Cost	0.023
Yearly Maintenance Cost	0.007

Table 4.6 Sensitivity analysis for the In situ Chlorination Process.

Input Variable	Sensitivity
Oxidant/Cyanide Ratio	0.740
Starting Cyanide Concentration	0.740
Oxidant Cost	0.501
Rinsing Rate	$1.000 \times ^{-0.20}$
Alkaline Cost	0.239
Yearly Labor Cost	0.168
Capital Costs	0.065
Electricity Cost	0.021
Yearly Maintenance Cost	0.007

Table 4.7 Sensitivity analysis for the Ex situ Biological Process.

Input Variable	Sensitivity
Capital Costs	0.443
Rinsing Rate	$1.025 \times ^{-0.38}$
Yearly Labor Cost	0.403
Starting Cyanide Concentration	0.101
Yearly Maintenance Cost	0.066
Phosphate Concentration	0.040
Phosphate Cost	0.040
Electricity Cost	0.033
Growth Medium Cost	0.014
Initial Inoculum Volume	0.014

Table 4.8 Sensitivity analysis for the INCO Process.

Input Variable	Sensitivity
Starting Cyanide Concentration	0.733
Oxidant/Cyanide Ratio	0.685
Alkaline Cost	0.316
Oxidant Cost	0.271
Yearly Labor Cost	0.224
Rinsing Rate	$1.000 \times ^{-0.20}$
Royalty	0.098
Capital Costs	0.059
Electricity Cost	0.024
Yearly Maintenance Cost	0.009

Table 4.9 Sensitivity analysis for the Ex situ Peroxide Process.

Input Variable	Sensitivity
Starting Cyanide Concentration	0.741
Oxidant/Cyanide Ratio	0.694
Oxidant Cost	0.694
Yearly Labor Cost	0.218
Rinsing Rate	$1.000 \times ^{-0.194}$
Capital Costs	0.056
Electricity Cost	0.023
Yearly Maintenance Cost	0.008

Table 4.10 Sensitivity analysis for the Ex situ Chlorination Process.

Input Variable	Sensitivity
Starting Cyanide Concentration	0.747
Oxidant/Cyanide Ratio	0.700
Oxidant Cost	0.474
Alkaline Cost	0.356
Yearly Labor Cost	0.214
Rinsing Rate	$1.000 \times ^{-0.19}$
Capital Costs	0.059
Electricity Cost	0.022
Yearly Maintenance Cost	0.008

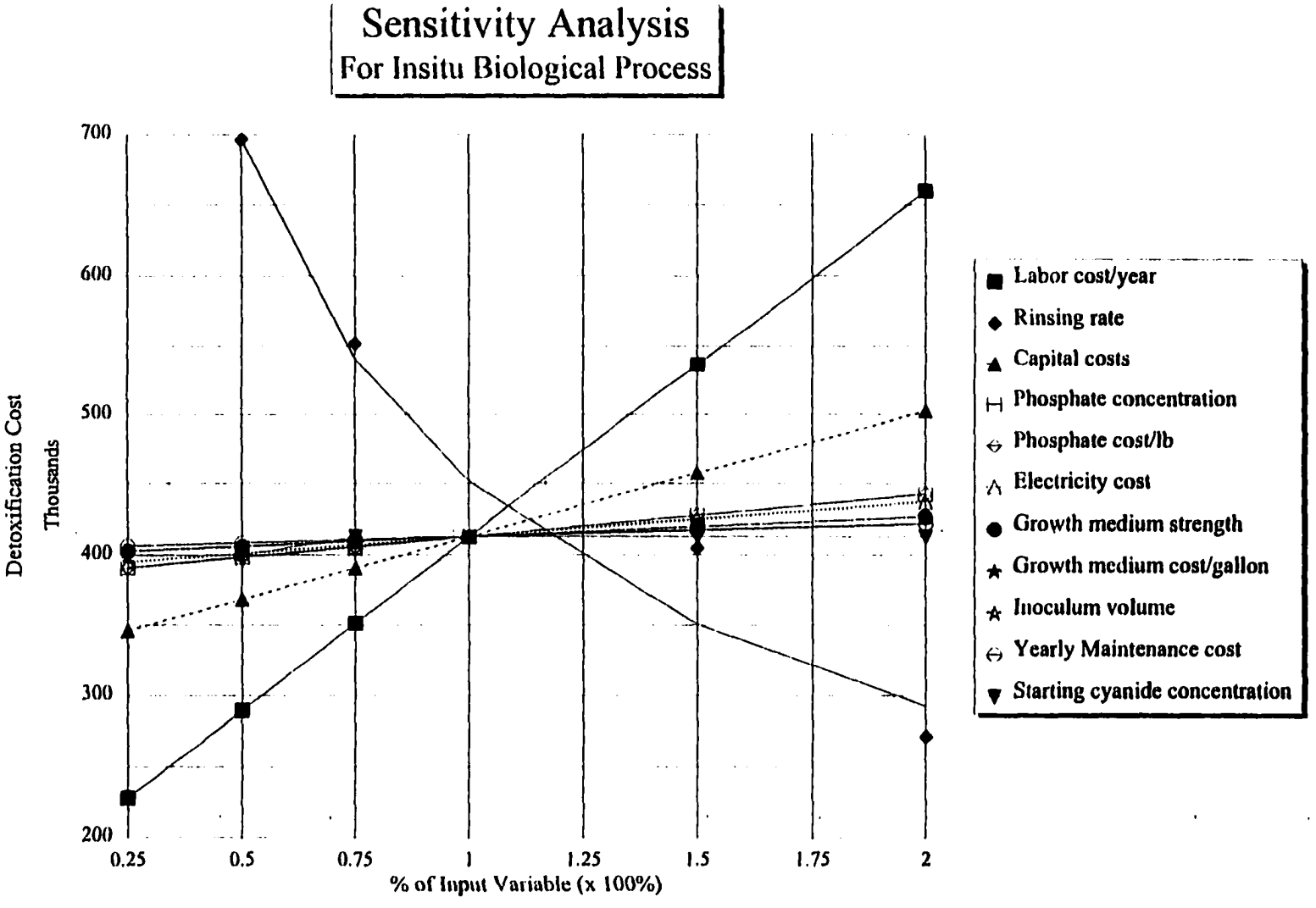


Figure 4.3 Sensitivity curves for the In situ Biological Process.

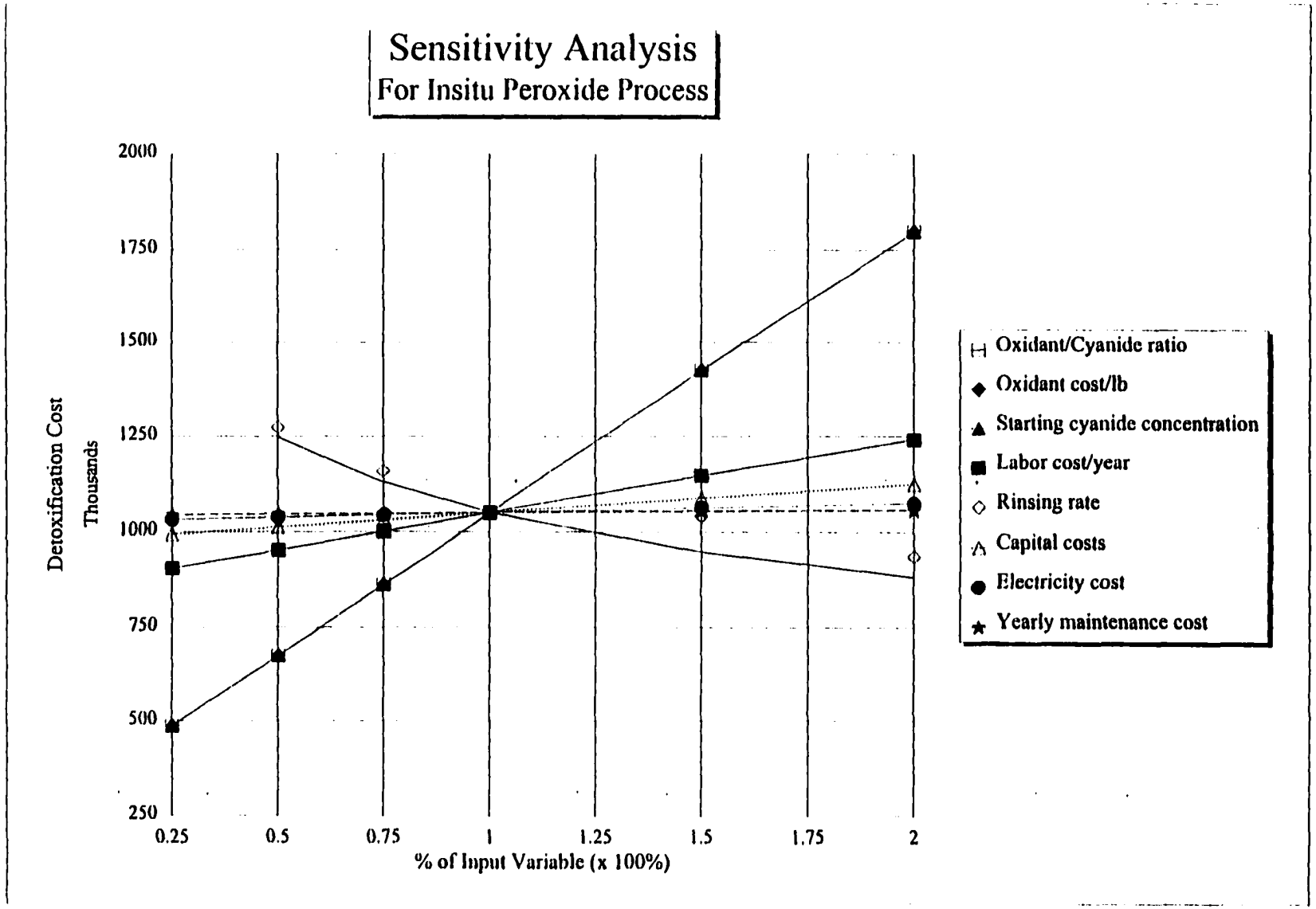


Figure 4.4 Sensitivity curves for the In situ Peroxide Process.

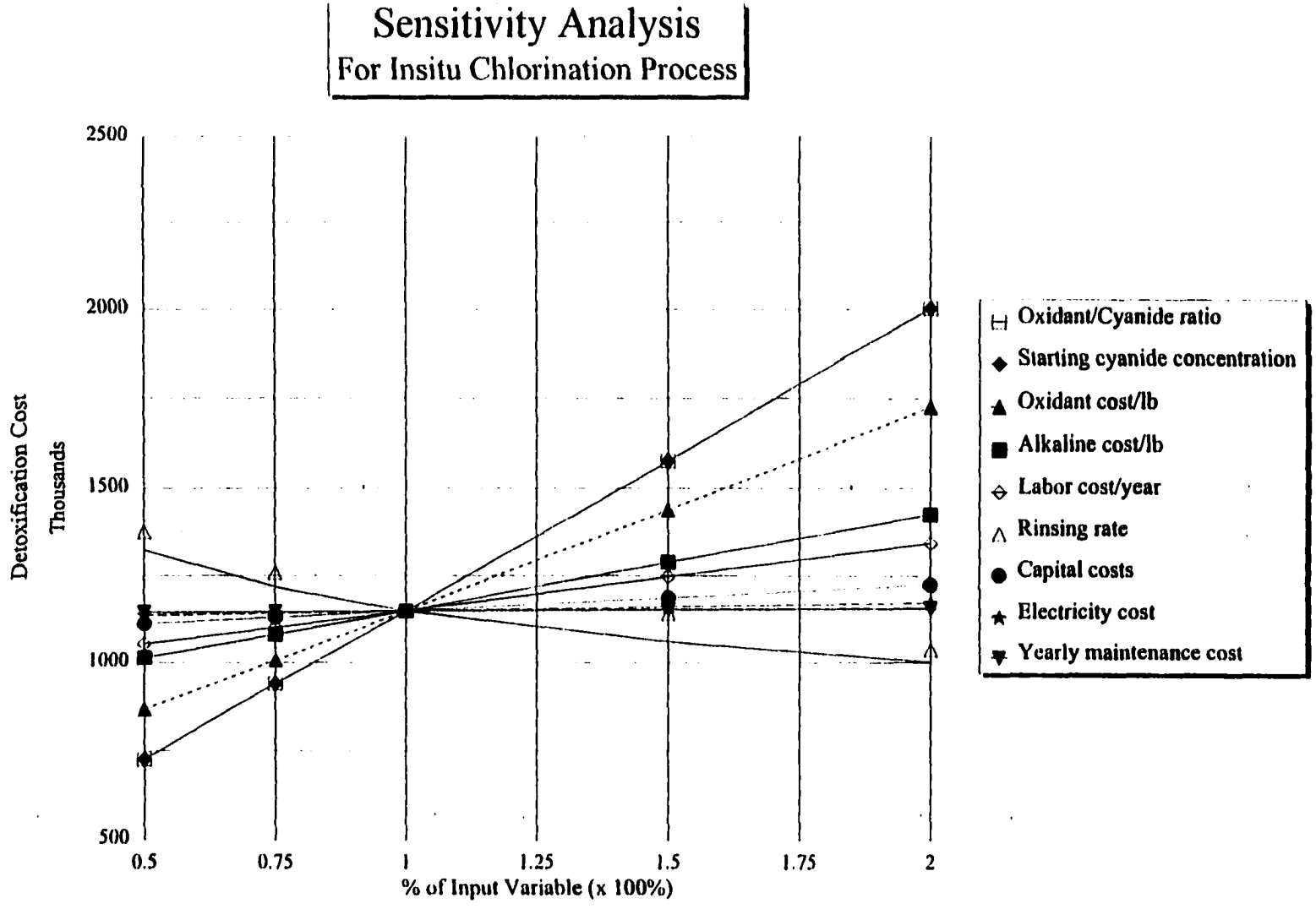


Figure 4.5 Sensitivity curves for the In situ Chlorination Process.

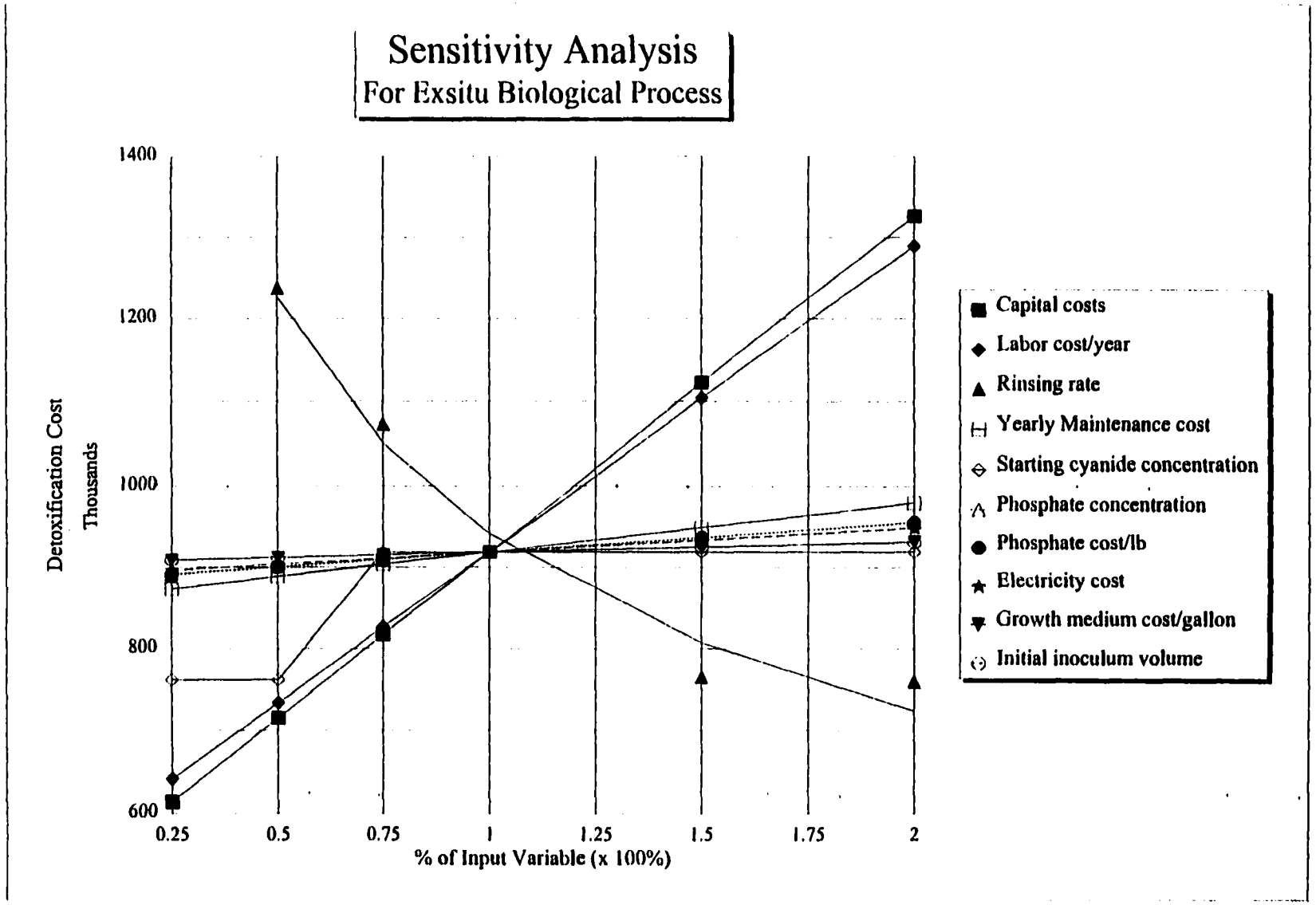


Figure 4.6 Sensitivity curves for the Ex situ Biological Process.

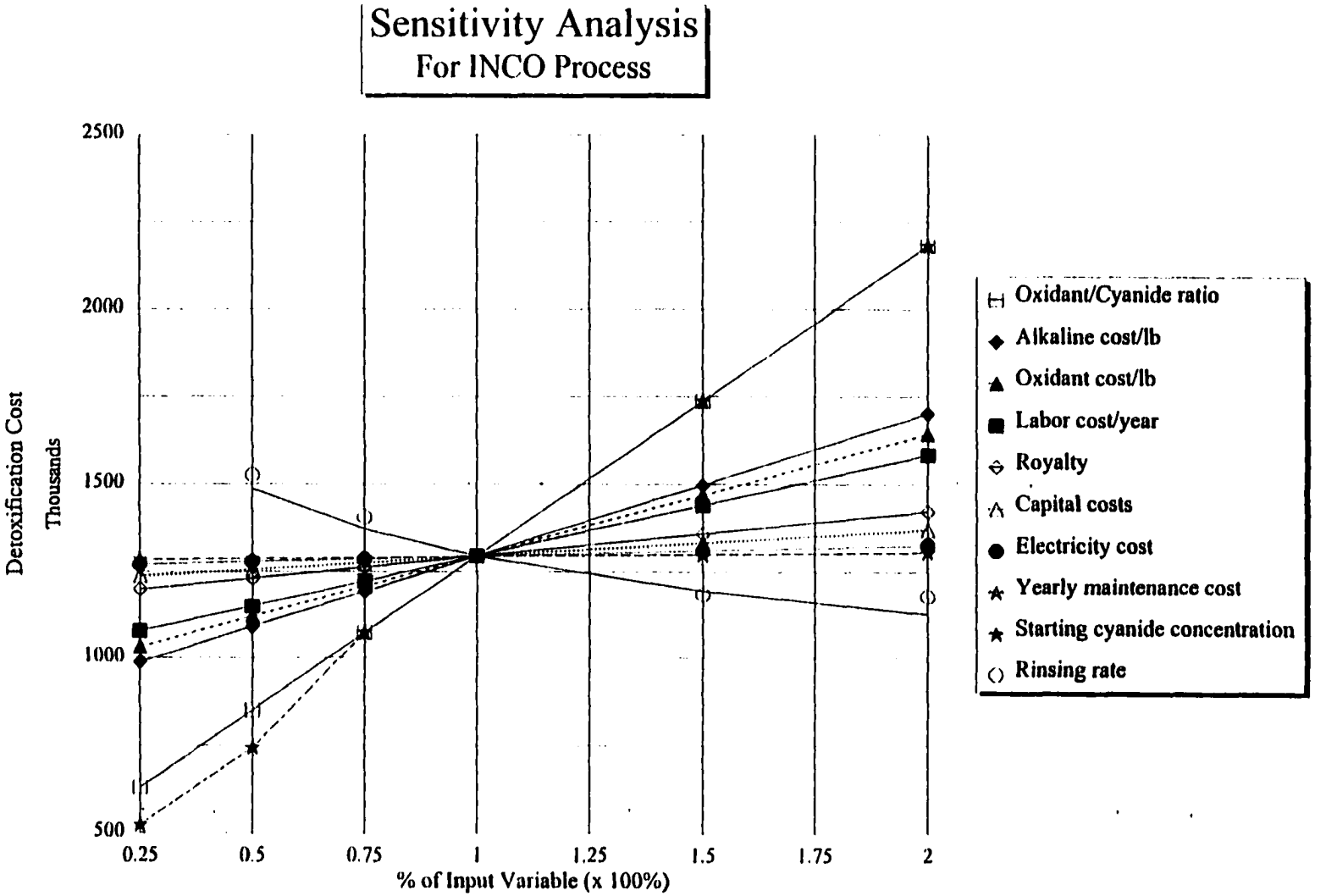


Figure 4.7 Sensitivity curves for the INCO air-SO_2 Process.

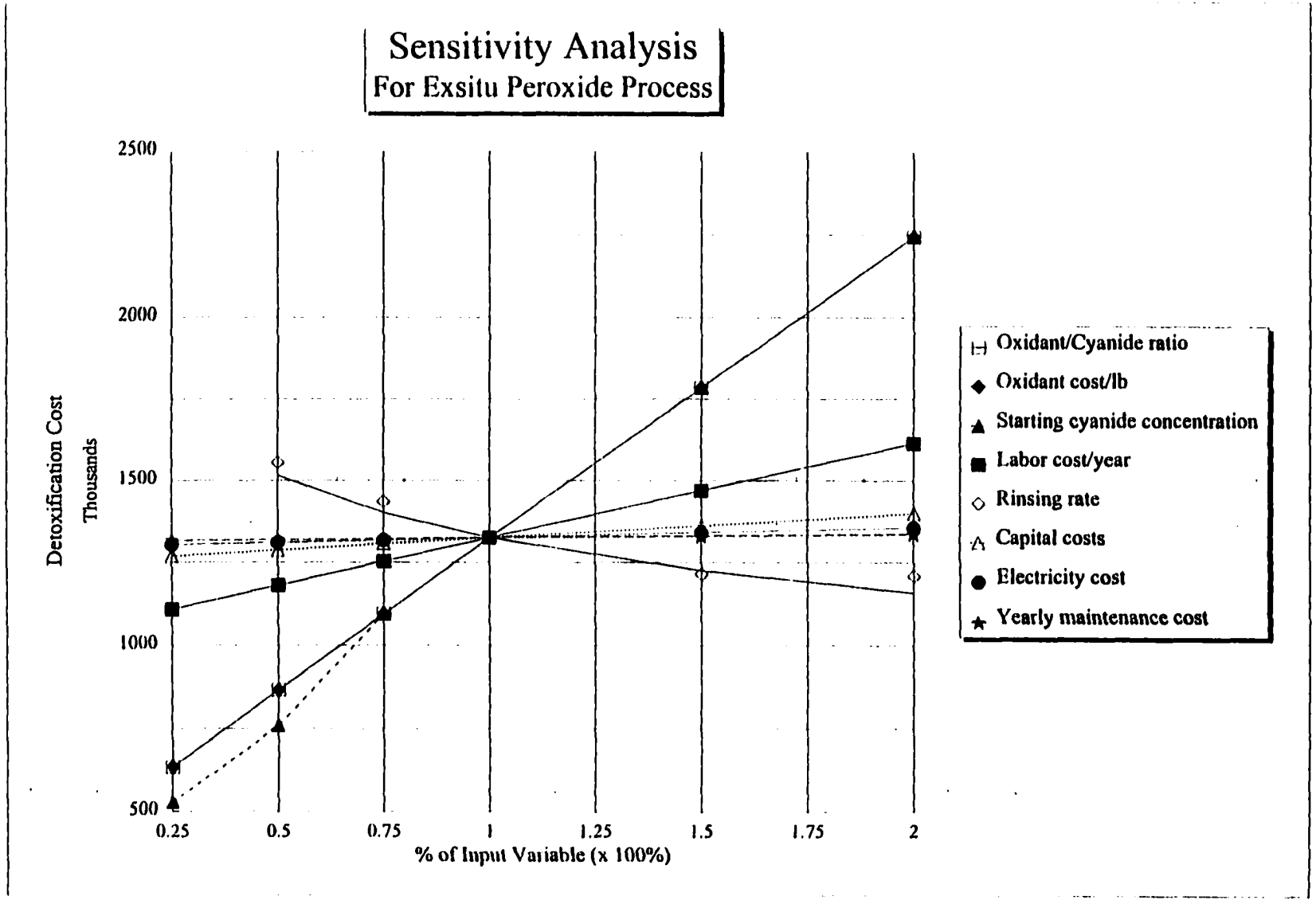


Figure 4.8 Sensitivity curves for the Ex situ Peroxide Process.

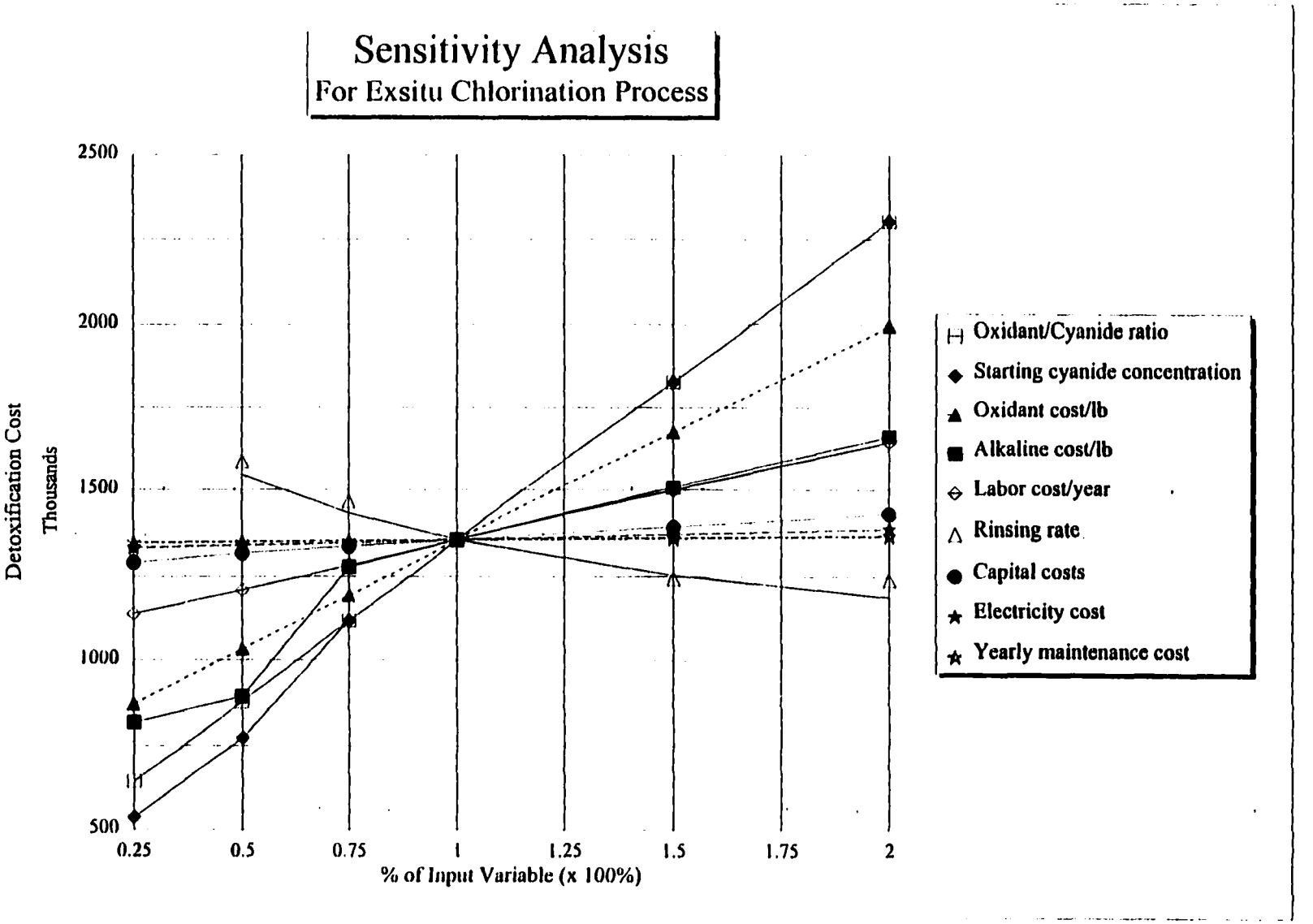


Figure 4.9 Sensitivity curves for the Ex situ Chlorination Process.

As expected, the in situ biological cyanide detoxification method was most sensitive to the rinsing rate, labor costs, and capital costs. Because the rinsing rate affected the number of seasons of rinsing, which in turn affected the labor costs, the rinsing rate had a large effect on the total cyanide detoxification cost. The chemical cyanide detoxification methods were most sensitive to the oxidant to cyanide ratio, oxidant cost, starting cyanide concentration, and rinsing rate. The ex situ biological cyanide detoxification method was most sensitive to the capital costs, rinsing rate, and labor costs.

One interesting result was obtained from the sensitivity analysis: when the starting cyanide concentration is below approximately 50 mg/l, chemical methods would be more cost effective than biological methods. This was mainly due to the somewhat fixed labor costs for the biological processes, and the chemical methods being highly sensitive to the amount and cost of the chemicals used.

4.4 Monte Carlo Simulation

The Monte Carlo simulation was developed during World War II by researchers working on the atomic bomb. The code name for these experiments was "Monte Carlo," after the city famous for its gambling (Software Bisque and Investment Evaluations Corp., 1990-91). Today, most simulations involving the selection of a random number from a known distribution are termed "Monte Carlo" simulations. Rather than using fixed values in the calculation of the total costs, random values are selected from a known distribution. Because it is a probability-based cost estimation

technique, the results not only provide the most likely cost, but also provide the probability of that cost occurring.

One problem with using a probability simulation is the accuracy of the input data. According to C. S. Park and G. P. Sharpe-Bette (1990), a triangular distribution (Figure 4.10) is often selected in the absence of data. For this research, the first step in defining the random variable x was to identify an interval from an optimistic low value, L, to a pessimistic high value, H, within which the random variable was contained with a probability close to one. For the triangular distribution, a subjective estimate of the most likely value (M_o) of the random variable was also chosen. The most likely value is the mode of the distribution x. The input values for each variable were chosen based on published data and personal experience. Park and Sharp-Bette

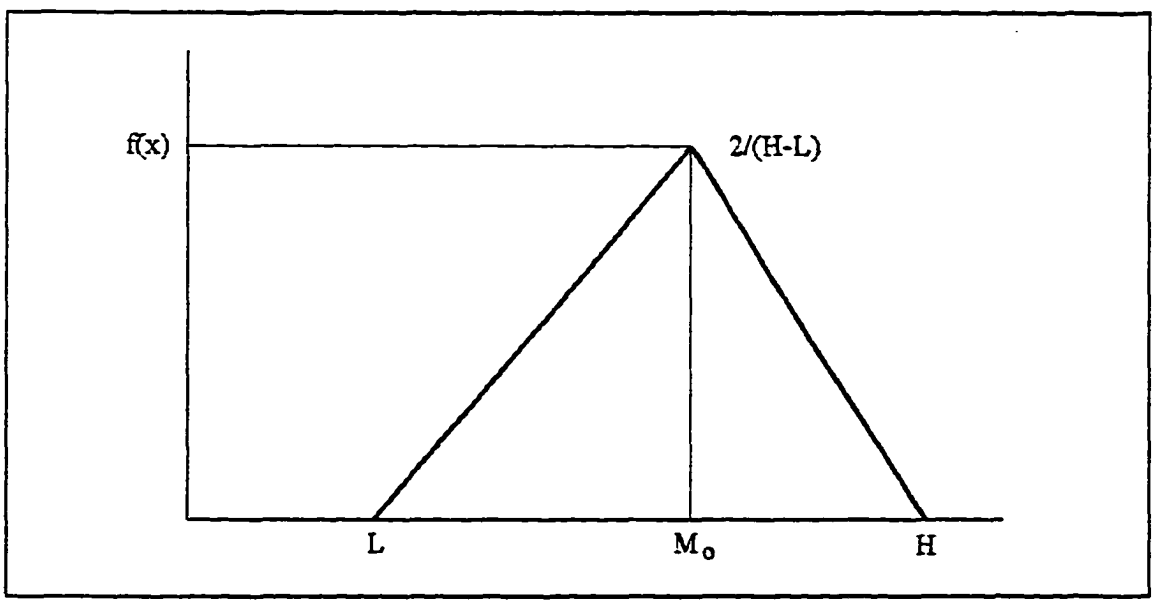


Figure 4.10 Triangular distribution (from Park, et al., 1990).

indicate the cumulative probability distribution, $F(x)$, is provided by either Equation 4.2 or 4.3.

$$F(x) = \begin{cases} \dfrac{(x-L)^2}{(H-L)(M_o-L)}, & L \le x < M_o \quad (4.2) \\ 1-\dfrac{(H-x)^2}{(H-L)(H-M_o)}, & M_o \le x \le H \quad (4.3) \end{cases}$$

They also provide Equations 4.4 and 4.5, for the expected value, $E(x)$, and variance, $Var(x)$, of the triangular distribution.

$$E(x) = \frac{L+M_o+H}{3} \quad (4.4)$$

$$Var(x) = \frac{1}{18}(L^2+M_o^2+H^2-LH-M_oH-M_oL) \quad (4.5)$$

Once the L, M_o, and H values were chosen, x was set equal to M_o, either Equation 4.2 or 4.3 rearranged, and the value of $F(x)$ determined. A random number generator was used to determine which equation would be used. The random number was then substituted for $F(x)$, and one of the equations was rearranged to yield the random cost, x, based on that random number. Every input variable listed in Table 4.11 with the corresponding L, M_o, and H values, was treated in this manner, and the total detoxification cost for each method was calculated 7,500 times. Histograms for the total detoxification cost each method were created are plotted in Figures 4.11-4.17.

Table 4.11 Input variables used in the Monte Carlo simulation.

Method	Variable	Lower Limit (L)	Most Likely (M)	Upper Limit (H)
In situ Biological	Capital Cost	$0	$89,366	$250,000
	Labor Cost	$25,000	$123,464	$300,000
	Growth Medium Strength	1%	10%	100%
	Growth Medium Cost	$0.10/gallon	$1.55/gallon	$3.10/gallon
	Initial Inoculum Volume	10,000 gallons	80,000 gallons	160,000 gallons
	Yearly Inoculum Volume	0 gallons	10,000 gallons	80,000 gallons
	Phosphate Concentration	5 mg/l	25 mg/l	100 mg/l
	Phosphate Cost	$0.10/pound	$0.95/pound	$1.90/pound
	Electricity Cost	$3,034/pore volume	$6,068/pore volume	$9,102/pore volume
	Maintenance Cost	$2,234/year	$4,468/year	$6,702/year
In situ Peroxide	Capital Cost	$0	$74,866	$250,000
	Labor Cost	$25,000	$96,464	$250,000
	Oxidant Cost	$0.10/pound	$0.90/pound	$1.50/pound
	Oxidant/Cyanide Ratio	3	7	11
	Electricity Cost	$3,034/pore volume	$6,068/pore volume	$9,102/pore volume
	Maintenance Cost	$1,871.50/year	$3,743/year	$5,614.50/year
In situ Chlorination	Capital Cost	$0	$74,451	$250,000
	Labor Cost	$25,000	$96,464	$250,000
	Oxidant Cost	$0.055/pound	$0.55/pound	$1.10/pound
	Alkaline Cost	$0.01/pound	$0.35/pound	$0.60/pound
	Oxidant/Cyanide Ratio	4	8	12
	Electricity Cost	$3,034/pore volume	$6,068/pore volume	$9,102/pore volume
	Maintenance Cost	$1,871.50/year	$3,743/year	$5,614.50/year
Ex situ Biological	Capital Cost	$203,658	$407,316	$1,000,000
	Labor Cost	$25,000	$123,464	$300,000
	Growth Medium Strength	10%	100%	300%
	Growth Medium Cost	$0.0655/gallon	$0.131/gallon	$0.262/gallon
	Initial Inoculum Volume	10,000 gallons	100,000 gallons	300,000 gallons
	Phosphate Concentration	5 mg/l	25 mg/l	100 mg/l
	Phosphate Cost	$0.10/pound	$0.95/pound	$1.90/pound

Method	Variable	Lower Limit (L)	Most Likely (M_o)	Upper Limit (H)
	Electricity Cost	$3,034/pore volume	$6,068/pore volume	$9,102/pore volume
	Maintenance Cost	$10,183/year	$20,366/year	$30,549/year
INCO Process	Capital Cost	$0	$76,042	$250,000
	Labor Cost	$25,000	$96,464	$250,000
	Oxidant Cost	$0.01/pound	$0.30/pound	$0.50/pound
	Alkaline Cost	$0.01/pound	$0.35/pound	$0.60/pound
	Oxidant/Cyanide Ratio	4	8	12
	INCO Royalty	$0.013/pound	$0.13/pound	$0.26/pound
	Electricity Cost	$3,130/pore volume	$6,260/pore volume	$9,390/pore volume
	Maintenance Cost	$1,901/year	$3,802/year	$5,703/year
Ex situ Peroxide	Capital Cost	$0	$74,866	$250,000
	Labor Cost	$25,000	$96,464	$250,000
	Oxidant Cost	$0.10/pound	$0.90/pound	$1.50/pound
	Oxidant/Cyanide Ratio	3	7	11
	Electricity Cost	$3,034/pore volume	$6,068/pore volume	$9,102/pore volume
	Maintenance Cost	$1,871.50/year	$3,743/year	$5,614.50/year
In situ Chlorination	Capital Cost	$0	$74,451	$250,000
	Labor Cost	$25,000	$96,464	$250,000
	Oxidant Cost	$0.055/pound	$0.55/pound	$1.10/pound
	Alkaline Cost	$0.01/pound	$0.35/pound	$0.60/pound
	Oxidant/Cyanide Ratio	4	8	12
	Electricity Cost	$3,034/pore volume	$6,068/pore volume	$9,102/pore volume
	Maintenance Cost	$1,871.50/year	$3,743/year	$5,614.50/year

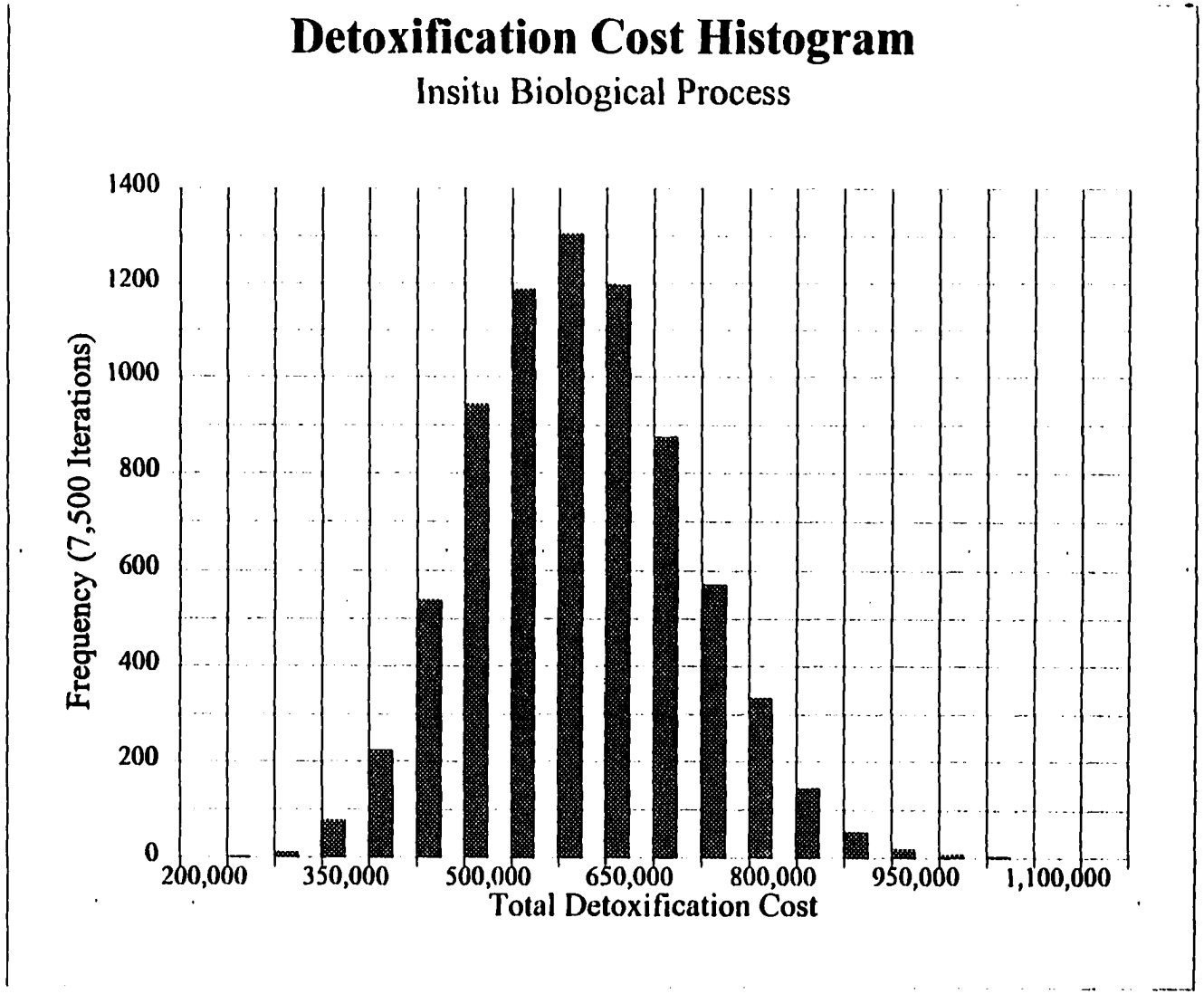


Figure 4.11 Histogram for the In situ Biological Process.

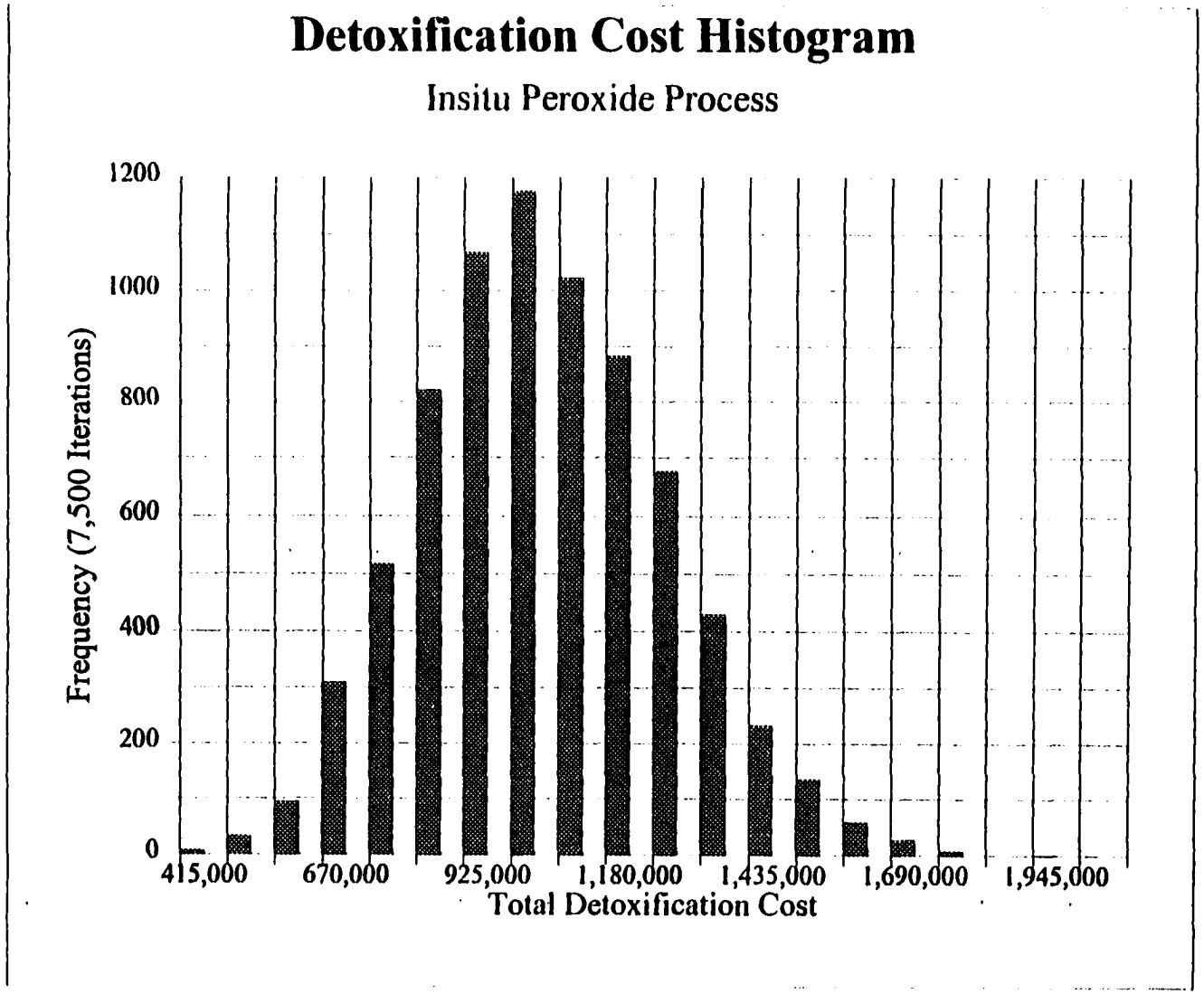


Figure 4.12 Histogram for the In situ Peroxide Process.

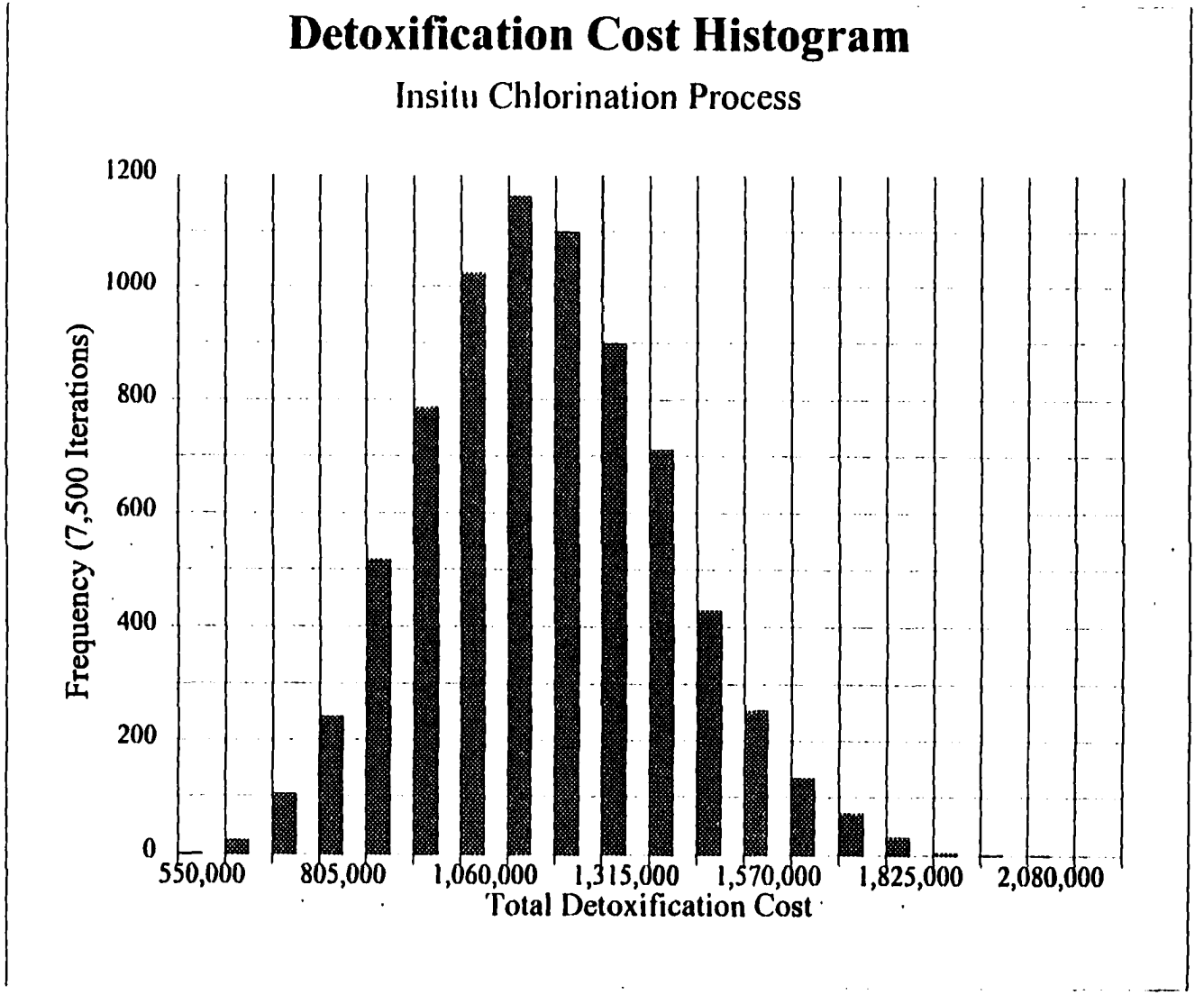


Figure 4.13 Histogram for the In situ Chlorination Process.

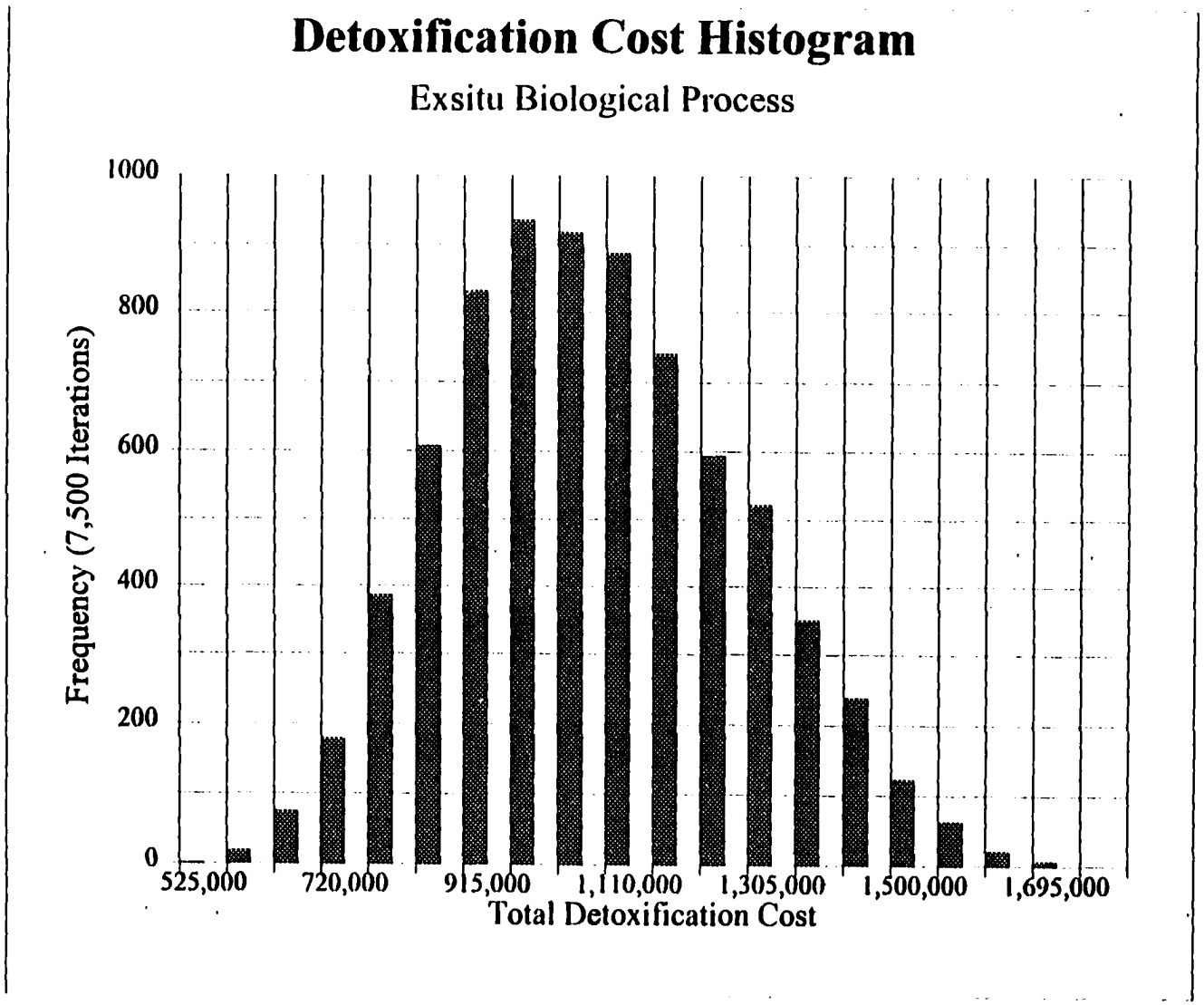


Figure 4.14 Histogram for the Ex situ Biological Process.

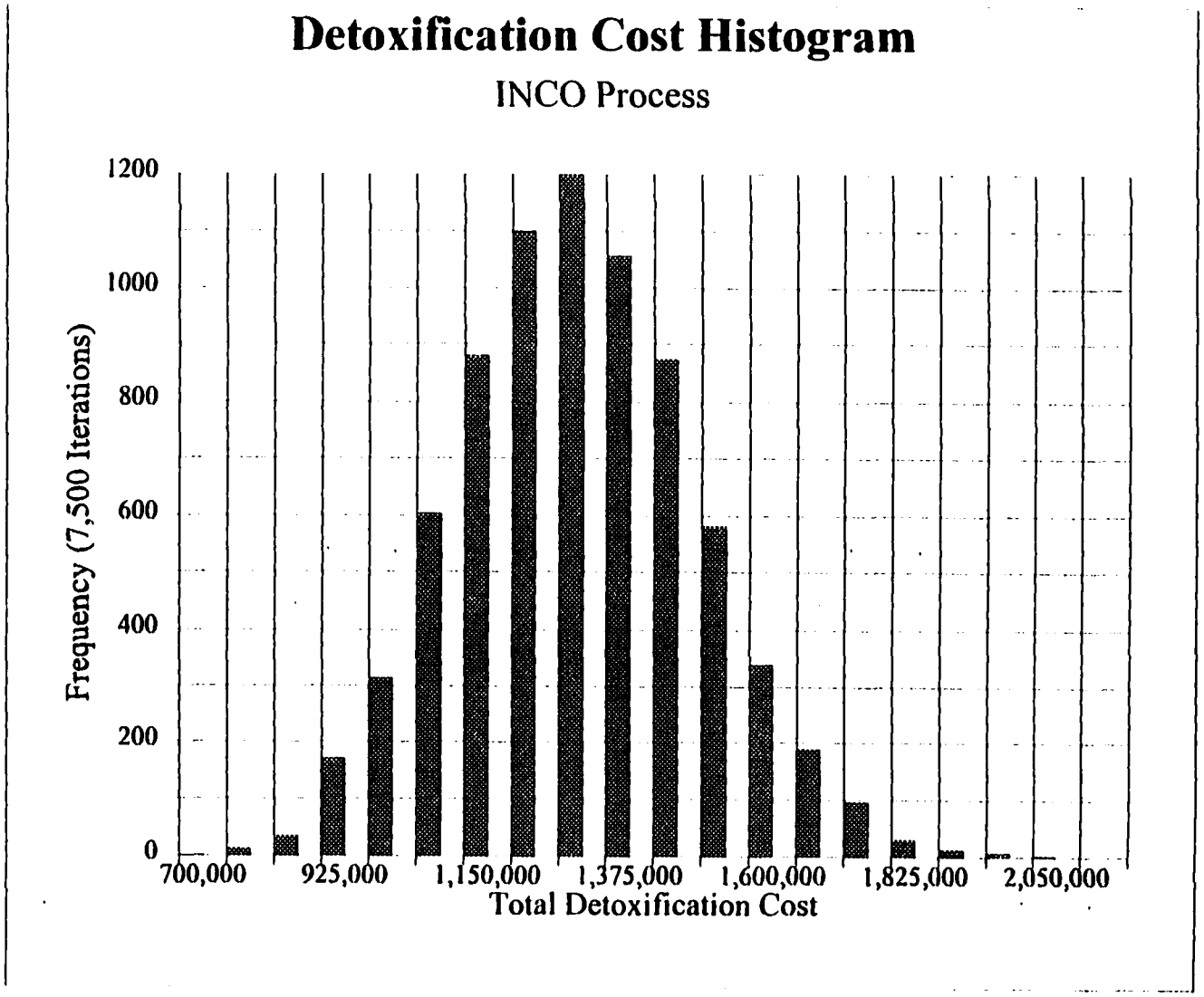


Figure 4.15 Histogram for the INCO air-SO_2 Process.

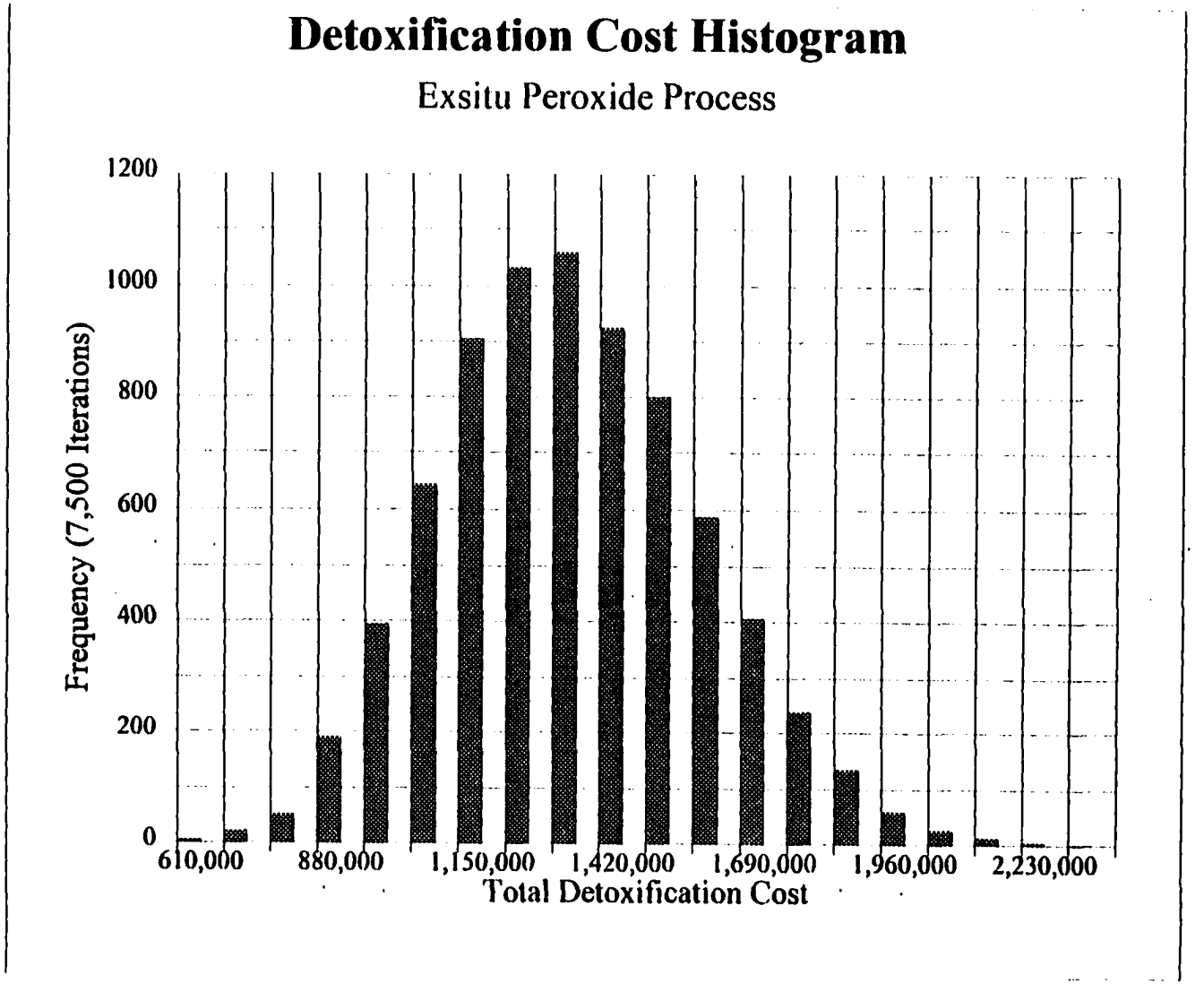


Figure 4.16 Histogram for the Ex situ Peroxide Process.

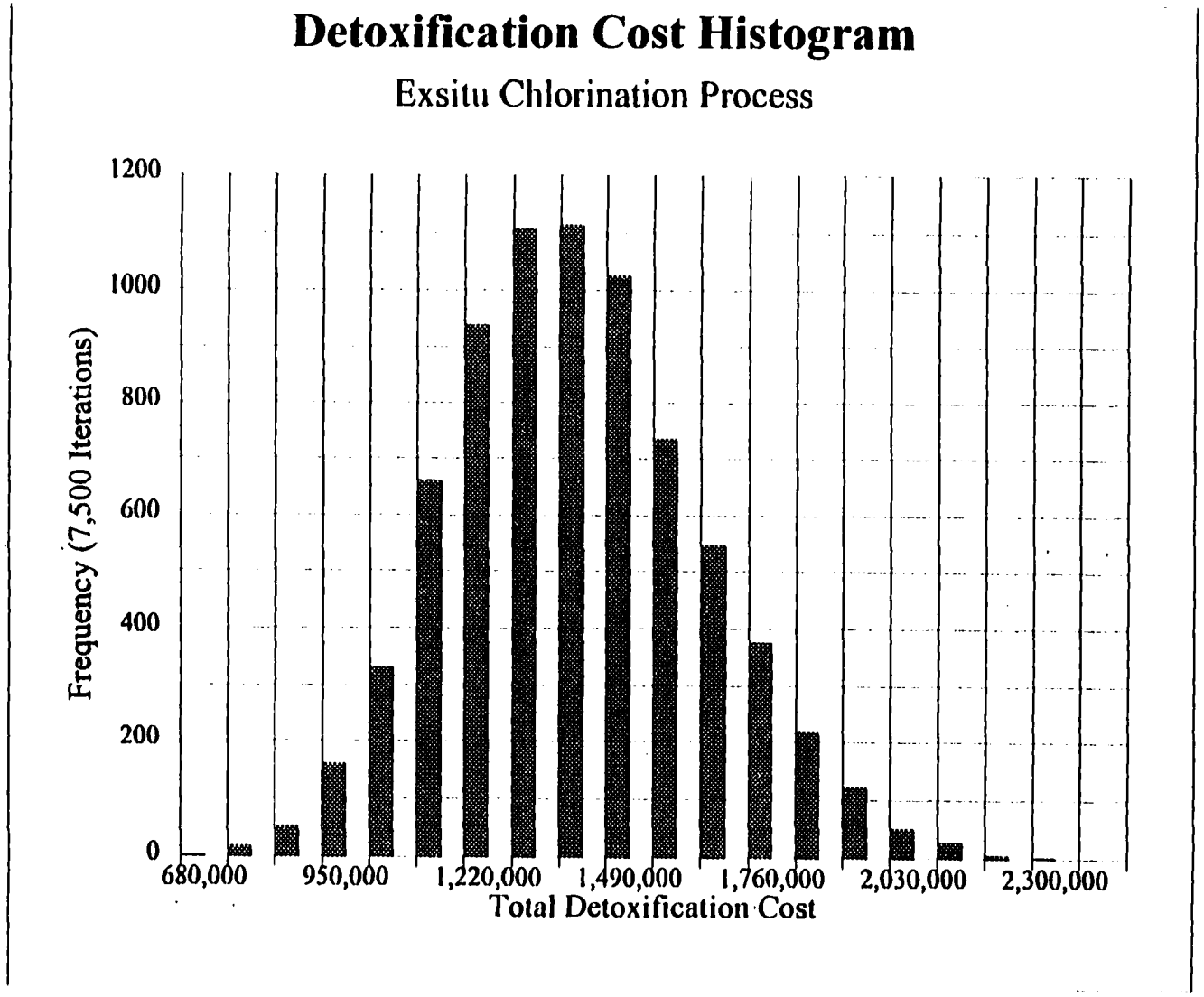


Figure 4.17 Histogram for the Ex situ Chlorination Process.

Table 4.12 lists the some of the statistics from the Monte Carlo simulation for each detoxification method.

Table 4.12 Statistics from the Monte Carlo simulation.

	Mean	Standard Deviation	95% Confidence Interval
In situ Biological	$633,058	$112,096	$408,866 - $857,250
In situ Peroxide	$1,086,362	$218,259	$649,844 - $1,522,880
Ex situ Biological	$1,108,299	$197,372	$713,555 - $1,503,043
In situ Chlorination	$1,231,101	$216,797	$797,507 - $1,664,695
INCO Air-SO_2	$1,344,903	$187,506	$969,891 - $1,719,915
Ex situ Peroxide	$1,385,841	$247,645	$890,551 - $1,881,131
Ex situ Chlorination	$1,451,824	$237,474	$976,876 - $1,926,772

There are differences between the mean total detoxification costs calculated using the Monte Carlo technique and the most likely costs provided in section 4.2.3. Because the distributions of the input variables greatly affects the histogram for the total detoxification cost, it was expected that the costs would vary using different estimation techniques. For every detoxification method, the mean calculated from the Monte Carlo simulation is higher than the most likely scenario. These differences could be from two causes: underestimation of the most likely costs, or use of overly pessimistic values for the upper limits (H) of the input variables. The means calculated from the Monte Carlo simulation for the chemical methods were within 8%

of the most likely costs, so it is unlikely that the first cause could be the result. Because the largest differences occurred with the two biological methods, and overly pessimistic values for H were selected for the growth medium strength, yearly inoculum volume, and phosphate concentration, the effects of the overly pessimistic values for H can clearly be seen.

For the in situ biological method, the Monte Carlo simulation indicated that there was only a 3% probability that the most likely cost would occur in the $375,000-$425,000 range. The ex situ biological method had an 11% probability that the most likely cost would occur in the $890,625-$939,375 range, which was better than the in situ biological method. The chemical methods had higher probabilities that the most likely cost would occur near the mean of the Monte Carlo simulation, in the 11%-15% range.

Overall, if the person conducting the economic analysis is unsure about the costs of the items or their quantities used, the Monte Carlo simulation seems to be a better method of cost estimation. As stated above, the distribution of the input variable also dramatically affects the overall costs. If the costs for a certain item are truly unknown, then it may be better to use a uniform distribution. The uniform distribution does have the drawback of increasing the standard deviation, which will increase the range of the confidence interval.

Because the costs are somewhat fixed for the biological processes, the Monte Carlo simulation seems to be a bit of an overkill, since a most likely cost needs to be chosen for one of the input values.

4.5 Optimization of Heap Detoxification

For the scenario analyzed, both in situ and ex situ biological detoxification have distinct economic advantages over the other processes investigated. As mentioned in section 4.1.1, for mine sites which are remote and have large shipping costs, in situ biological detoxification would have an even greater advantage. However, neither in situ nor ex situ biological detoxification are suited to every site: Sites which have toxins in the wastewater such as silver or arsenic, sites where the starting cyanide concentration is low, sites where the starting cyanide concentration is too high for the bacteria to tolerate, or sites which have a high risk of exposure will all be less suited to biological detoxification.

Proper thought should be given to the cyanide detoxification method during the mine planning stage. The mine planner may find it advantageous to reduce the cyanide concentration in the leach solution in order to reduce the detoxification costs. This may, however, reduce the overall gold recovery and recovery rate from the heap. This may not be an acceptable alternative for projects that are economically marginal. To reduce the possibility of a process not performing properly, a series of column studies should be always be conducted on ore from the mine during the planning stage. These column studies will help determine the heap operating parameters such as the optimum cyanide concentration during leaching, recovery rate, and overall gold recovery.

As shown in this dissertation, benefits may be realized when an in situ cyanide detoxification process is chosen over an ex situ process. The in situ chemical

techniques studied all involved the oxidation of cyanide to less or non-toxic forms. These same oxidizing chemicals are also used for pre-cyanidation treatment of ores to increase the overall gold recovery by oxidizing a portion of the recalcitrant sulfides. Using these chemicals in situ in a heap before leaching may have the added benefit of oxidizing recalcitrant sulfides which encapsulate gold particles, making more gold available for leaching, thereby enhancing the overall gold recovery.

During the first three months Thompson operated the biological detoxification system on the 1.3 million ton heap at the Yellow Pine mine, Hecla reported a release of gold from the heap that had a value greater than the cost of the heap detoxification (Mattison, P., 1992). The mechanism for the gold release is not fully understood, but oxidation of recalcitrant sulfides in the ore as well as oxidation of the cyanide-metal complexes may play an important role in this release. The production of biosurfactants by the bacteria may lower the surface tension in the ore by enhancing the wetability may also play an important role. Both of these areas have a great potential for future research.

An additional area which should be researched is the combination of detoxification methods. In situ biological techniques require the input of oxygen in order to oxidize the cyanide. Oxygen is also required for in situ biological detoxification of hydrocarbons. In the past, this oxygen requirement was met by injecting dilute hydrogen peroxide or bubbling oxygen through solution before injection into the contaminated area. Using this reasoning, there should be an increase in the cyanide detoxification rate with the combination of the in situ biological and in

situ peroxide processes. If the bacteria are in an oxygen deficient environment, it will aid in their growth or, if there is excess oxygen, then it will oxidize the cyanide naturally.

CHAPTER FIVE

5.0 CONCLUSIONS

This dissertation has discussed and analyzed biological cyanide detoxification tests that were conducted at the University of Alaska Fairbanks and elsewhere. This research work by Dr. Arps at UAF and others has proven the merits of biological cyanide detoxification and in particular, a strain of *Pseudomonas pseudoalcaligenes*.

Collection and isolation of cyanide degraders is neither an easy nor quick process. As shown in this work, sometimes natively occurring cyanide degraders cannot be isolated from the mine site, and suitable strains must be located elsewhere. On the slim chance that neither native nor non-native suitable strains cannot be found, a population of organisms may be induced to degrade cyanide (Ingvorsen, et al., 1991). One difficulty in the isolation of cyanide degraders is competition for food from native cyanide-tolerant organisms. Work on soil samples from the Ryan Lode site isolated several bacterial colonies that could tolerate cyanide in the growth medium, but none were found that degraded cyanide.

Over-winter survival experiments indicated that approximately 5-20% of the bacterial population in the heap agglomerate samples were viable and survived the winter and subsequent wait in a coldroom. In addition, bacteria colonized the agglomerate samples where nutrients were available.

This dissertation also discussed and analyzed the engineering aspects of cyanide destruction using seven different techniques. For the scenario analyzed, in

situ biological detoxification has several advantages over the other six types of processes analyzed. One advantage is that a separate detoxification pond is not required for in situ biological detoxification. Because a pond or reaction vessel is required to destroy the cyanide using the INCO air-SO_2 method, the by-products of the destruction process, namely the metal hydroxides, are left in the pond. When the cyanide is degraded in place, the by-products of the cyanide oxidation remain in the heap, and the problems of waste by-product disposal are reduced.

Another advantage to using in situ detoxification over ex situ processes is the elimination of the longest step in the removal of the cyanide, the diffusion and lixiviation of the cyanide from the heap solids.

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APPENDIX A: PICRIC ACID METHOD

THE DIRECT SPECTROPHOTOMETRIC DETERMINATION OF CYANIDE WITH PICRIC ACID REAGENT

P. IAMARINO

JRGRL June 1, 1989

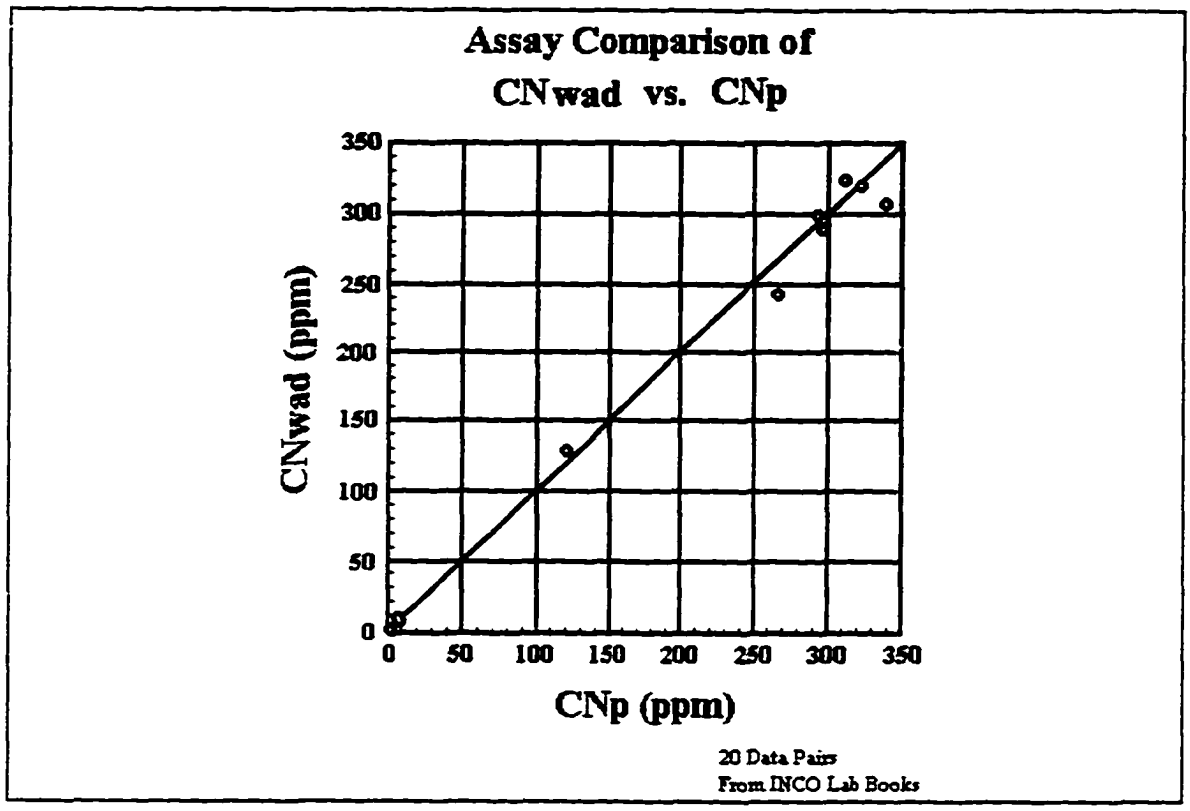
1. OUTLINE

Free cyanide and weak-acid dissociable cyanide reacts with the picric acid reagent to produce an orange colour which can be measured spectrophometrically at 520 nm or can be estimated by eye. As with all spectrophotometric methods, standards must be prepared for comparison purposes.

Chemically speaking, the dissolved alkali metal picrate is converted by cyanide into the coloured salt of isopurpuric acid and its concentration is measured. For determination of cyanide below 0.1 ppm extractive preconcentration techniques can be used which are described in the original method. The presence of a small amount of nickel in the analyzed solutions has a positive effect on the overall performance of the method.

2. APPLICATION

The method is suitable for the determination of weak acid dissociable cyanide (CN_{WAD}) in effluents from flotation mills, gold cyanidation mills and electroplating shops. Since the detection limit for this procedure is 0.26 ppm CN_{WAD} it is especially



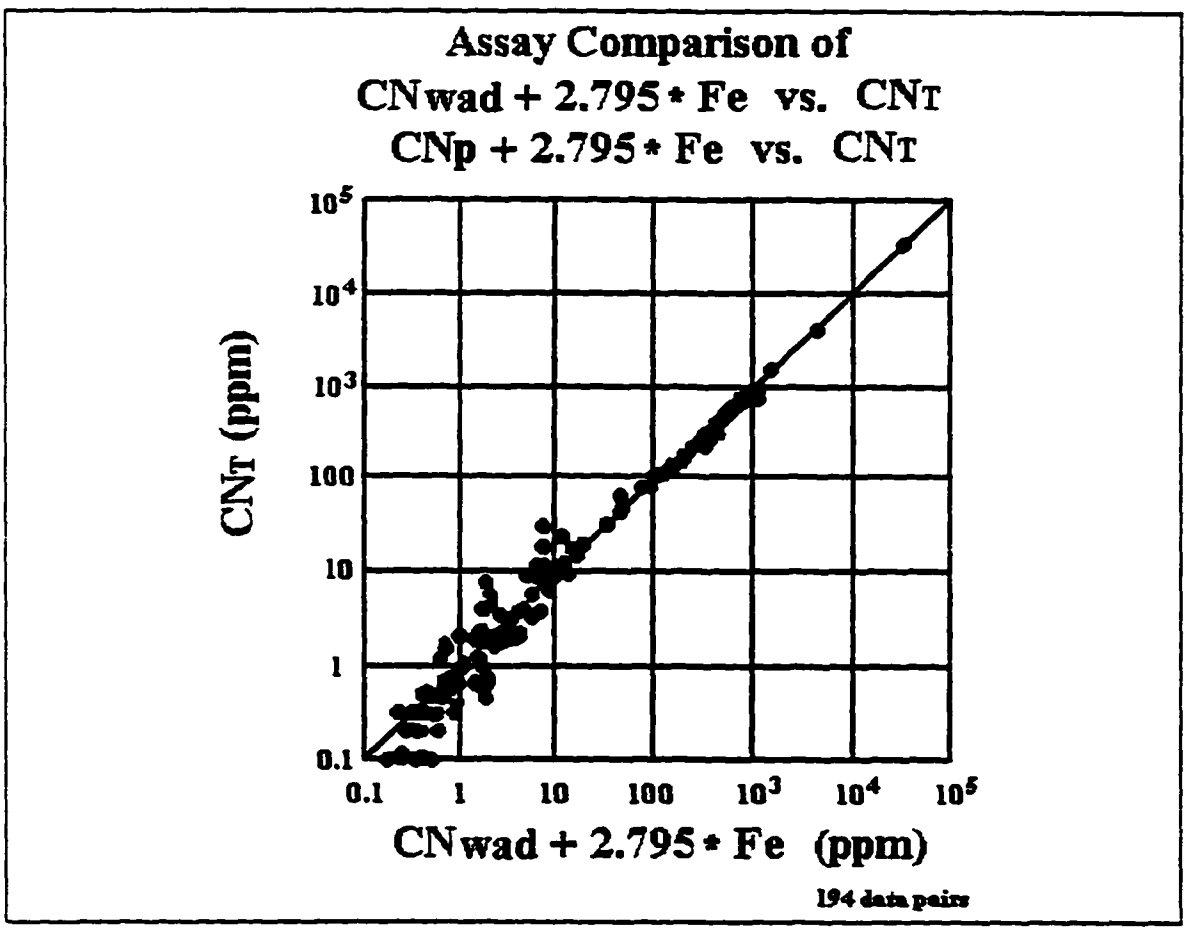
useful for monitoring cyanide discharges to the environment. Below is a comparison of the results from the CN_{WAD} distillation method (CN_D) and that obtained with the picric acid method (CN_P) described herein. It is apparent that CN_D is essentially the same as CN_P though it is useful to retain the distinction between the methods.

The reduction of picric acid is affected by free cyanide only. Cyanide tied up in copper, nickel, zinc or cadmium complexes is liberated by metathesis with diethylenetriaminepentaacetic acid (DTPA) or ethylenediaminetetraacetic acid (EDTA). Iron-cyanide complexes, cobalt-cyanide complexes, gold-cyanide complexes, and silver-cyanide complexes do not react, leaving their portion of cyanide undetermined. Usually only iron cyanide is present in any significant concentration so one can

calculate total cyanide (CN_T) with one of the two formulae:

$$CN_T = CN_P + 2.795 \times Fe$$
$$CN_T = CN_{WAD} + 2.795 \times Fe$$

As the concentrations of CN_P or CN_{WAD} and Fe approach zero, the formula becomes less exacting since a ligand other than cyanide might be present to keep the iron (Fe) in solution. Below is a comparison of results from the total cyanide distillation method (CN_T) and that obtained with above formulae:



The direct spectrophotometric mode method allows for the accurate measurement of 20 - 300 μg CN^- in a sample aliquot of up to 75 mL. For samples containing greater than 600 ppm CN_p, dilute with distilled water. The table below should be used as a guide to determine the sample aliquot to be used:

Cyanide Concentration in Aliquot (ppm CN_p)	Aliquot (mL)
0.2 - 4	75
0.8 - 12	25
2 - 30	10
4 - 60	5
20 - 300	1
40 - 600	0.5

3. INTERFERENCES

Thiocyanate, cyanate and thiosulfate ions have no adverse effects and can be tolerated at levels normally occurring in gold mill effluents. Sulfide is a source of interference, 0.1 mg S^{2-} being equivalent to 0.025 mg CN^-. If present, sulfide ions can be readily removed by the addition of lead salts. However, it is unlikely that mill effluents would contain sulfide at levels large enough to significantly interfere in the cyanide determination. Sulfide particles which contact the picric acid reagent because of improper filtering of a gold bearing slurry, will also cause the S^{2-} interferences.

The method requires a close control of pH since it affects the colour intensity produced by the cyanide-picric acid reaction. The most intense coloration result at pH

9.0 - 9.5. For maximum sensitivity and a good reproducibility of analytical results, the picric acid reagent solution should therefore be buffered. In the present procedure a mixture of sodium tetraborate and carbonate as well as DTPA itself serve this purpose. DTPA is preferred to EDTA due to more favorable values of acid ionization constants and stability constants of some metal chelates.

The method highly recommended for monitoring the effluent of the INCO SO_2-Air cyanide removal process since it is simple to do (one the picric acid reagent has been prepared) and any procedural errors will result in a result biased high; whereas, distillation methods will be biased low with any procedural errors. If the SO_2-Air cyanide destruction process has lost its catalytic activity, an reagent due to and SO_3^{2-} interference (unoxidized SO_2 in solution). The interference can be removed by adding $CaCl_2$ which will precipitate the SO_3^{2-} as $CaSO_2$. The precipitate must be filtered out prior to using the spectrophotometer.

4. SAFETY PRECAUTIONS

Solutions of picric acid (trinitrophenol) are safe in ordinary laboratory use. However, in dry form the acid and especially some of its salts have explosive properties. This requires that all picric acid solutions be thoroughly washed down a sink with water. Spills must be carefully wiped up. Picric acid has the tendency of staining the skin, and wearing protective hand gloves is therefore recommended. Glass stained by picric acid is best washed with methanol or acetone.

5. REAGENTS

Buffered Picric Acid Reagent

Dissolve 40 g of diethylenetriaminepentaacetic acid and 16 g of NaOH in 900 - 950 ml of water. Next dissolve, in the order given, 6 g of picric acid, 14 g of anhydrous $Na_2B_4O_7$ (or 27 g of $Na_2B_4O_7x10H_2O$) and 8 g of anhydrous Na_2CO_3. The pH of this solution is 8.7, and would increase to 9.0 on four-fold dilution. After reacting with cyanide the final pH should be 9.2 - 9.3.

NOTE: In the preparation of the above reagent, 37 g of EDTA with 8 g of NaOH may be substituted for the 40 g of DTPA and 16 g of NaOH.

Nickel Solution, approximately 100 mg/l Ni

Dissolve 0.22 g of $NiSO_4x6H_2O$ and 1 g of NaCl in 500 ml of water.

Standard Cyanide Solution, 1000 µg/mL CN^-

Dissolve 2.503 g of KCN and 1 g of KOH or NaOH in water and dilute to 1 litre. Make further dilutions as necessary for the calibration purpose.

6. PROCEDURE

6.1 Transfer into a 150 ml beaker a suitable volume of sample solution which contains 1 - 300 µg of CN^-. Add 1 ml of nickel solution, swirl, and dilute with water to about 70 mL. Measure about 70 ml of water in another beaker, add 1 ml of nickel solution and carry through the procedure as the reagent blank. Add 25.0 ml of

buffered picric acid reagent to each beaker (Note 1) and heat for 30 minutes on a hotplate with surface temperature adjusted to 160°C (320°F) or 1 to 2 minutes in a microwave oven (Note 2). Cool the solutions to room temperature, transfer to 100 mL volumetric flasks and dilute to volume.

6.2 Direct Spectrophometry (20 -300 μg CN^-): Measure the absorbance of solutions more deeply colored than the reagent blank at 520 nm using the reagent blank as the reference (Note 3).

7. CALIBRATION

Into 150 mL beakers, pipette aliquots of the standard cyanide solution containing 25, 50, 100, 200 and 300 μg CN-. Add 1 mL nickel solution to each of them, mix, and dilute to about 70 mL with water. Add 25.0 mL of buffered picric acid reagent and proceed as described in the procedure 6.1, and 6.2 or 6.3. Always measure absorbance against the respective reagent blank. Plot the absorbance readings vs. μg CN^- added in the aliquots of the standard cyanide solution, and construct the calibration graph.

8. CALCULATION

Convert the absorbance reading of the aqueous solution or the extract into micrograms of cyanide using the calibration graph. Calculate the cyanide concentration in the original sample solution as follows:

$$mg/l\ CN^- = \frac{A}{B}$$

where A = µg CN^- found from the graph, and B = volume, in ml, of the sample solution used for the analysis.

NOTES:

1. A white precipitate of calcium carbonate might separate from samples containing large quantities of calcium. This dissolved by addition of 0.1 - 0.2 g of EDTA disodium salt.
2. The solution should reach a temperature close to the boiling point but should not be allowed to boil for any length of time. Alternately, the analysis can be carried out in 250 mL conical flasks which are then immersed in a boiling water bath for a period of 20 minutes.

   Microwave heating is the latest innovative approach for color development. Instructions and precautions are provided under the addendum "SAMPLE COLOR DEVELOPMENT USING A MICROWAVE OVEN".
3. The absorbance of the reagent blank usually varies between 0.006 - 0.009 (520 nm, slit width 0.03 mm, 1-cm glass cell).

REMARK: The procedure is a modification of V.J. Zatka method (JRGRL, November 1980) which was also a modification of the method by a D.J. Barkley and J.C. Ingles, Report R 221, CANMET, (February 1970).

ADDENDUM

January 9, 1990

SAMPLE COLOR DEVELOPMENT USING A MICROWAVE OVEN

Recently, it was discovered that a microwave oven can be used to minimize the time requirement for color development. This approach should be followed only when the procedure and results are to be used as a monitoring tool rather than an analytical tool. It should also be understood that certain precautionary measures must be taken by the operator when the microwave approach is used.

Major and minor spills must be thoroughly cleaned immediately before operating the microwave again. This prevent formation of dry picric acid salts which are potentially dangerous.

Heating time depends on type and power output of the microwave oven. Sample color development should be monitored closely by the operator.

The following procedure will allow safe and quick color development.

1. Place sample in the microwave centered on the heating platform.
2. Set heating time for 2 minutes, close the door and begin heating.
3. Heat for 40 seconds, open the microwave door and swirl the sample carefully. The sample should be fairly warm. The swirling prevents hot spots from forming throughout the solution that would cause bumping and possibly a spill.
4. Close the oven door and again heat the solution just to boiling. Stop

the heating and with caution using hand protection (solution is hot) swirl the flask again to homogenize the solution.

5. Place the flask back into the oven and heat for a further 5 to 20 seconds until the solution just starts to reboil.
6. Turn the microwave oven off, carefully remove the sample solution from the oven and allow it to sit for 3 to 4 minutes before placing the flask in a cooling water bath. Cool sample to room temperature before measuring color intensity.
7. The operator must turn the microwave off very quickly when he observes the first signs of boiling in step 4 and 5, otherwise the solution will bump and spill.

APPENDIX B: EPA METHOD 335.2

CYANIDE, TOTAL

Method 335.2 (Titrimetric; Spectrophotometric)

STORET NO. 00720

1. Scope and Application

   1.1 This method is applicable to the determination of cyanide in drinking, surface, and saline waters, and domestic, and industrial wastes.

   1.2 The titration procedure using silver nitrate with p-dimethylamino-benzal-rhodanine indicator is used for measuring concentrations of cyanide exceeding 1 mg/L (0.25 mg/250 ml of absorbing liquid)

   1.3 The colorimetric procedure is used for concentrations below 1 mg/L of cyanide and is sensitive to about 0.02 mg/L.

2. Summary of Method

   2.1 The cyanide is hydrocyanic acid (HCN) is released from cyanide complexes by means of a reflux-distillation operation and absorbed in a scrubber containing sodium hydroxide solution. The cyanide ion in the absorbing solution is then determined by volumetric titration or colorimetrically.

   2.2 In the colorimetric measurement, the cyanide is converted to cyanogen chloride (CNCl) by reaction with chloramine-T at a pH less than 8 without hydrolyzing to the cyanate. After the reaction is complete, color is formed on the addition of pyridine-pyrazolone or pyridine-

barbituric acid reagent. The absorbance is read at 620 nm when using pyridine-pyrazolone or 578 nm for pyridine-barbituric acid. To obtain colors of comparable intensity, it is essential to have the same salt content in both the sample and the standards.

2.3 The titrimetric measurement uses a standard solution of silver nitrate to titrate cyanide in the presence of a silver sensitive indicator.

3. Definitions

3.1 Cyanide is defined as cyanide ion and complex cyanides converted to hydrocyanic acid (HCN) by reaction in a reflux system of a mineral acid in the presence of magnesium ion.

4. Sample Handling and Preservation

4.1 The sample should be collected in plastic or glass bottles of 1 liter or larger size. All bottles must be thoroughly cleansed and thoroughly rinsed to remove soluble material from containers.

4.2 Oxidizing agents such as chlorine decompose most of the cyanides. Test a drop of the sample with potassium iodide-starch test paper (KI-starch paper); a blue color indicates the need for treatment. Add ascorbic acid, a few crystals at a time, until a drop of sample produces no color on the indicator paper. Then add an additional 0.06 g of ascorbic acid for each liter of sample volume.

4.3 Samples must be preserves with 2 ml of 10 N sodium hydroxide (NaOH) per liter of sample (pH $\geq$ 12) at the time of collection.

4.4 Samples should be analyzed as rapidly as possible after collection. If storage is required, the samples should be stored in a refrigerator or in an ice chest filled with water and ice to maintain temperature at 4°C.

5. Interferences

5.1 Interferences are eliminated or reduced by using the distillation procedure described in Procedure 8.1 and 8.3.

5.2 Sulfides adversely affect the colorimetric and titration procedures. Samples that contain hydrogen sulfide, metal sulfides or other compounds that may produce hydrogen sulfide during the distillation should be distilled by the optional procedure described in Procedure 8.2. The apparatus for this procedure is shown in Figure 3.

5.3 Fatty acids will distill and form soaps under the alkaline titration conditions, making the end point almost impossible to detect.

5.3.1 Acidify the sample with acetic acid (1+ 9) to pH 6.0 to 7.0.

Caution: This operation must be performed in the hood and the sample left there until it can be made alkaline again after the extraction has been performed.

5.3.2 Extract with iso-octane, hexane, or chloroform (preference in order named) with a solvent volume equal to 20% of the sample

volume. One extraction is usually adequate to reduce the fatty acids below the interference level. Avoid multiple extractions or a long contact time at low pH in order to keep the loss of HCN at a minimum. When the extraction is completed, immediately raise the pH of the sample to above 12 with NaOH solution.

5.4 High results may be obtained for samples that contain nitrate and/or nitrite. During the distillation nitrate and nitrite will form nitrous acid which will react with some organic compounds to form oximes. These compounds formed will decompose under test conditions to generate HCN. The interference of nitrate and nitrite is eliminated by pretreatment with sulfamic acid.

6. Apparatus

6.1 Reflux distillation apparatus such as shown in Figure 1 or Figure 2. The boiling flask should be of 1 liter size with inlet tube and provision for condenser. The gas absorber may be a Fisher-Milligan scrubber.

6.2 Microburet, 5.0 ml (for titration).

6.3 Spectrophotometer suitable for measurements at 578 nm or 620 nm with a 1.0 cm cell or larger.

6.4 Reflux distillation apparatus for sulfide removal as shown in Figure 3. The boiling flask same as 6.1 The sulfide scrubber may be a Wheaton Bubber #709682 with 29/42 joints, size 100 ml. The air inlet tube

should not be fritted. The cyanide absorption vessel should be the same as the sulfide scrubber. The air inlet tube should be fritted.

6.5 Flow meter, such as Lab Crest with stainless steel float (Fisher 11-164-50).

7. Reagents

7.1 Sodium hydroxide solution, 1.25 N: Dissolve 50 g of NaOH in distilled water, and dilute to 1 liter with distilled water.

7.2 Lead acetate: Dissolve 30 g of $Pb(C_2H_3O_2)x3H_2O$ in 950 ml of distilled water. Adjust the pH to 4.5 with acetic acid. Dilute to 1 liter.

7.3 Sulfuric acid; 18 N: Slowly add 500 ml of concentrated H_2SO_4 to 500 ml of distilled water.

7.4 Sodium dihydrogenphosphate, 1 M: dissolve 138 g of $NaH_2PO_4xH_2O$ in 1 liter of distilled water. Refrigerate this solution.

7.5 Stock cyanide solution: Dissolve 2.51 g of KCN and 2 g KOH in 900 ml of distilled water. Standardize with 0.0192 N $AgNO_3$. Dilute to appropriate concentration so that 1 ml = 1 mg CN.

7.6 Standard cyanide solution, intermediate: Dilute 100.0 ml of stock (1 ml = 1 mg CN) to 1000 ml with distilled water (1 ml = 100.0 μg).

7.7 Working standard cyanide solution: Prepare fresh daily by diluting 100.0 ml of intermediate cyanide solution to 1000 ml with distilled water and store in a glass stoppered bottle. 1 ml = 10.0 μg CN.

7.8 Standard silver nitrate solution, 0.0192 N: Prepare by crushing

approximately 5 g $AgNO_3$ crystals and drying to constant weight at 40°C. Weigh out 3.2647 g of dried $AgNO_3$, dissolve in distilled water, and dilute to 1000 ml (1 ml = 1 mg CN).

7.9 Rhodanine indicator: Dissolve 20 mg of p-dimethyl-amino-benzal-rhodanine in 100 ml of acetone.

7.10 Chloramine T solution: Dissolve 1.0 g of white, water soluble Chloramine T in 100 ml of distilled water and refrigerate until ready to use. Prepare fresh daily.

7.11 Color Reagent - One of the following may be used:

7.11.1 Pyridine-Barbituric Acid Reagent: Place 15 g of barbituric acid in a 250 ml volumetric flask and add just enough distilled water to wash the sides of the flask and wet the barbituric acid. Add 75 ml of pyridine and mix. Add 15 ml of conc. HCl, mix, and cool to room temperature. Dilute to 250 ml with distilled water and mix. This reagent is stable for approximately six months if stored in a cool, dark place.

7.11.2 Pyridine-pyrazolone solution:

7.11.2.1 3-Methyl-1-phenyl-2-pyrazolin-5-1 reagent, saturated solution: Add 0.25 g of 3-Methyl-1-phenyl-2-pyrazolin-5-1 to 50 ml of distilled water, heat to 60°C with stirring. Cool to room temperature.

7.11.2.2 3,3'-Dimethyl-1, 1'-diphenyl-[4,4'-bi-2 pyrazoline]-5-5'-

di-1 (bispyrazolone): Dissolve 0.01 g of bispyrazolone in 10 ml of pyridine.

7.11.2.3 Pour solution (7.11.2.1) through non-acid-washed filter paper. Collect the filtrate. Through the same filter paper pour solution (7.11.2.2) collecting the filtrate in the same container as filtrate from (7.11.2.1). Mix until the filtrates are homogeneous. The mixed reagent develops a pink color but this does not affect the color production with cyanide if used within 24 hours of preparation.

7.12 Magnesium chloride solution: Weigh 510 g of $MgCl_2x6H_2O$ into a 1000 ml flask, dissolve and dilute to 1 liter with distilled water.

7.13 Sulfamic acid.

8. Procedure

8.1 For samples without sulfide.

8.1.1 Place 500 ml of sample, or an aliquot diluted to 500 ml in the 1 liter boiling flask. Pipet 50 ml of sodium hydroxide (7.1) into the absorbing tube. If the apparatus in Figure 1 is used, add distilled water until the spiral is covered. Connect the boiling flask, condenser, absorber and trap in the train. (Figure 1 or 2).

8.1.2 Start a stream of air entering the boiling flask by adjusting the vacuum source. Adjust the vacuum so that approximately two

bubbles of air per second enters the boiling flask through the air inlet tube. Proceed to 8.4

8.2 For samples that contain sulfide.

8.2.1 Place 500 ml of sample, or an aliquot diluted to 500 ml in the 1 liter boiling flask. Pipet 50 ml of sodium hydroxide (7.1) into the absorbing tube. Add 25 ml of lead acetate (7.2) to the sulfide scrubber. Connect the boiling flask, condenser, scrubber and absorber in the train. The flow meter is connected to the outlet tube of the cyanide absorber.

8.2.8 Start a stream of air entering the boiling flask by adjusting the vacuum source. Adjust the vacuum so that approximately 1.5 liters per minute enters the boiling flask through the air inlet tube. The bubble rate may not remain constant while heat is being applied to the flask. It may be necessary to readjust the air rate occasionally. Proceed to 8.4.

8.3 If samples contain NO_3 and or NO_2 add 2 g of sulfamic acid solution (7.13) after the air rate is set through the air inlet tube. Mix for 3 minutes prior to addition of H_2SO_4.

8.4 Slowly add 50 ml 18 N sulfuric acid (7.3) through the air inlet tube. Rinse the tube with distilled water and allow the airflow to mix the flask contents for 3 min. Pour 20 ml of magnesium chloride (7.12) into the air inlet and wash down with a stream of water.

8.5 Heat the solution to boiling. Reflux for one hour. Turn off heat and continue the airflow for at least 15 minutes. After cooling the boiling flask, disconnect absorber and close off the vacuum source.

8.6 Drain the solution from the absorber into a 250 ml volumetric flask. Wash the absorber with distilled water and add the washings to the flask. Dilute to the mark with distilled water.

8.7 Withdraw 50 ml or less of the solution from the flask and transfer to a 100 ml volumetric flask. If less than 50 ml is taken, dilute to 50 ml with 0.25 N sodium hydroxide solution. Add 15.0 ml of sodium phosphate solution (7.4) and mix.

8.7.1 Pyridine-barbituric acid method: Add 2 ml of chloramine T (7.10) and mix. See Note 1. After 1 to 2 minutes, add 5 ml of pyridine-barbituric acid solution (7.11.1) and mix. Dilute to mark with distilled water and mix again. Allow 8 minutes for color development then read absorbance at 578 nm in a 1 cm cell within 15 minutes.

8.7.2 Pyridine-pyrazolone method: Add 0.5 ml of chloramine T (7.10) and mix. See Note 1 and 2. After 1 to 2 minutes, add 5 ml of pyridine-pyrazolone solution (7.11.2) and mix. Dilute to mark with distilled water and mix again. After 40 minutes, read absorbance at 620 nm in a 1 cm cell.

NOTE 1: Some distillates may contain compounds that have a

chlorine demand. One minute after the addition of chloramine T, test for residual chlorine with KI-starch paper. If the test is negative, add an additional 0.5 ml of chloramine T. After one minute, recheck the sample.

NOTE 2: More than 0.5 ml of chloramine T will prevent the color from developing with pyridine-pyrazolone.

8.8 Standard curve for samples without sulfide.

8.8.1 Prepare a series of standards by pipeting suitable volumes of standard solution (7.7) into 250 ml volumetric flasks. To each standard add 50 ml of 1.25 N sodium hydroxide and dilute to 250 ml with distilled water. Prepare as follows:

ml of Working Standard Solution (1 ml = 10 µg CN)	Conc. µg of CN per 250 ml
0	Blank
1.0	10
2.0	20
5.0	50
10.0	100
15.0	150
20.0	200

8.8.2 It is not imperative that all standards be distilled in the same manner as the samples. It is recommended that at least two

standards (a high and low) be distilled and compared to similar values on the curve to insure that the distillation technique is reliable. If distilled standards do not agree within ± 10% of the undistilled standards, the analyst should find the cause of the apparent error before proceeding.

8.8.3 Prepare a standard curve by plotting absorbance of standard vs. cyanide concentrations.

8.8.4 To check the efficiency of the sample distillation, add an increment of cyanide from either the intermediate standard (7.8) or the working standard (7.9) to 500 ml of sample to insure a level of 20 ug/1. Proceed with the analysis as in Procedure (8.1.1).

8.9 Standard curve for samples with sulfide.

8.9.1 It is imperative that all standards be distilled in the same manner as the samples. Standards distilled by this method will give a linear curve, but as the concentration increases, the recovery decreases. It is recommended that at least 3 standards be distilled.

8.9.2 Prepare a standard curve by plotting absorbance of standard vs. cyanide concentrations.

8.10 Titrimetric method.

8.10.1 If the sample contains more than 1 mg/L of CN, transfer the

distillate or a suitable aliquot diluted to 250 ml, to a 500 ml Erlenmeyer flask. Add 10-12 drops of the benzal-rhodanine indicator.

8.10.2 Titrate with standard silver nitrate to the first change in color from yellow to brownish-pink. Titrate a distilled water blank using the same amount of sodium hydroxide and indicator as in the sample.

8.10.3 The analyst should familiarize himself with the end point of the titration and the amount of indicator to be used before actually titrating the samples.

9. Calculation

9.1 If the colorimetric procedure is used, calculate the cyanide, in µg/liter, in the original sample as follows:

$$CN,\ \mu/l = \frac{A \times 1{,}000}{B} \times \frac{50}{C}$$

where:

A = ug CN read from standard curve

B = ml of original sample for distillation

C = ml taken for colorimetric analysis

9.2 Using the titrimetric procedure, calculate concentration of CN as follows:

$$CN,\ \mu/l = \frac{(A - B) \times 1{,}000}{ml\ of\ original\ sample} \times \frac{50}{ml\ of\ aliquot\ titrated}$$

where:

A = volume of $AgNO_3$ for titration of sample.

B = volume of $AgNO_3$ for titration of blank.

10. Precision and Accuracy

10.1 In a single laboratory (EMSL), using mixed industrial and domestic waste samples at concentrations of 0.06, 0.13, 0.28 and 0.62 mg/L CN, the standard deviation were ±0.005, ±0.007, ±0.031 and ±0.094, respectively.

10.2 In a single laboratory (EMSL), using mixed samples at concentrations of 0.28 and 0.62 mg/L CN, recoveries were 85% and 102%, respectively.

Bibliography

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4. Standard Methods for the Examination of Water and Wastewater, 14th Edition, p 367 and 370, Method 413B and D (1975).

5. Egekeze, J.O., and Oehne, F.W., "Direct Potentiometric Determination of Cyanide in Biological Materials," J. Analytical Toxicology, Vol. 3, p. 119, May/June 1979.

6. Casey, J.P., Bright, J.W., and Helms, B.D., "Nitrosation Interference in Distillation Tests for Cyanide," Gulf Coast Waste Disposal Authority, Houston, Texas.

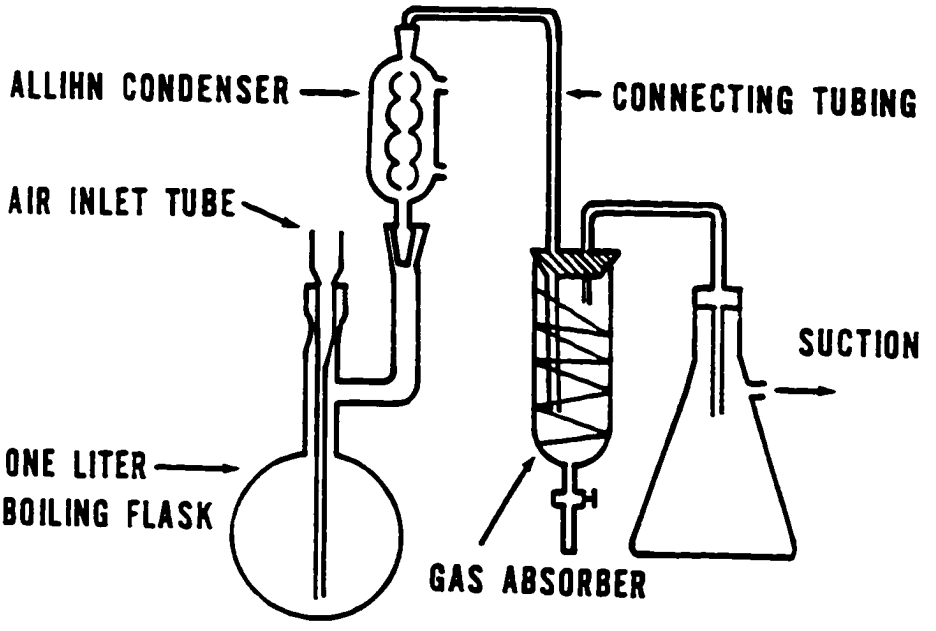

FIGURE 1

CYANIDE DISTILLATION APPARATUS

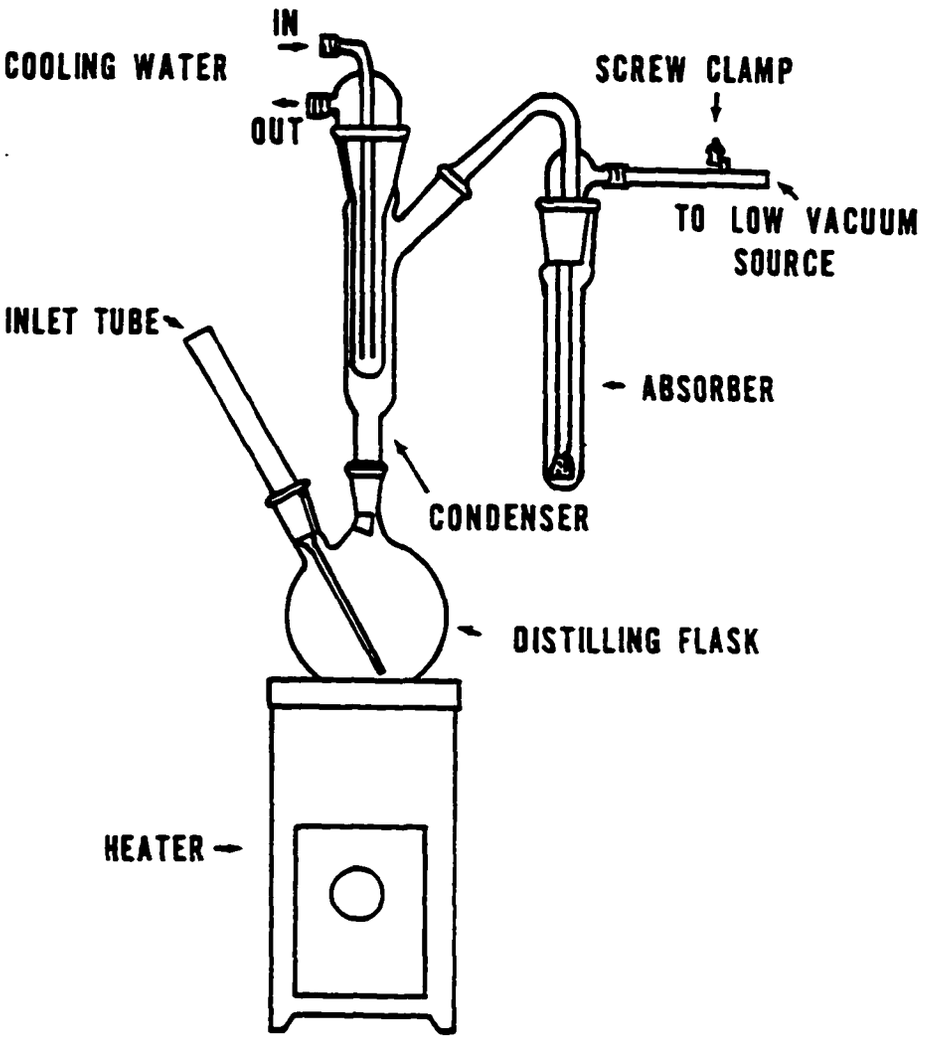

FIGURE 2

CYANIDE DISTILLATION APPARATUS

APPENDIX C: NORTHERN TESTING LABS RESULTS

NORTHERN TESTING LABORATORIES, INC.

1330 INDUSTRIAL AVENUE, FAIRBANKS, ALASKA 99701, (907) 456-3116 • FAX 456-3125
2505 FAIRBANKS STREET, ANCHORAGE, ALASKA 99503, (907) 277-8378 • FAX 274-9845

University of Alaska Fairbanks
M.I.R.L.
206 Brooks Bldg
PO Box 755800
Fairbanks AK 99775
Attn: Edwin Kroeger

Report Date: 09/22/94

Date Arrived: 08/29/94
Date Sampled: 07/18/94
Time Sampled: -
Collected By: Edwin

MDL = Method Detection Limit

Our Lab #: F141795
Location/Project: School
Your Sample ID: Test Heap
Sample Matrix: Soil
Comments:

* Flag Definitions
B = Below Regulatory Min.
H = Above Regulatory Max.

Method	Parameter	Units	Results *	MDL	Date Prepared	Date Analyzed
EPA 160.3	Solids	%	89.6	1.0		09/19/94
EPA 335.2	Total Cyanide	mg/dry kg	185	13		09/07/94
SM 412H	Cyanide, Weak & Dissociable	mg/dry kg	35	9		09/13/94

Reported By: James H. Johnson
Vice-President

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PO Box 755800
Fairbanks AK 99775
Attn: Edwin Kroeger

Report Date: 09/22/94

Date Arrived: 08/29/94
Date Sampled: 08/02/94
Time Sampled: -
Collected By: Edwin

MDL = Method Detection Limit

Our Lab #: F141794
Location/Project: School
Your Sample ID: Test Heap
Sample Matrix: Soil
Comments:

* Flag Definitions
B = Below Regulatory Min.
H = Above Regulatory Max.

Method	Parameter	Units	Results *	MDL	Date Prepared	Date Analyzed
EPA 160.3	Solids	%	87.5	1.0		09/19/94
EPA 335.2	Total Cyanide	mg/dry kg	166	15		09/07/94
SM 412H	Cyanide, Weak & Dissociable	mg/dry kg	39	5		09/13/94

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Vice-President

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Report Date: 09/22/94
Date Arrived: 08/29/94
Date Sampled: 08/08/94
Time Sampled: -
Collected By: Edwin

MDL = Method Detection Limit

Our Lab #: F141793
Location/Project: School
Your Sample ID: Test Heap
Sample Matrix: Soil
Comments:

* Flag Definitions
B = Below Regulatory Min.
H = Above Regulatory Max.

Method	Parameter	Units	Results *	MDL	Date Prepared	Date Analyzed
EPA 160.3	Solids	%	86.6	1.0		09/19/94
EPA 335.2	Total Cyanide	mg/dry kg	76	19		09/07/94
SM 412H	Cyanide, Weak & Dissociable	mg/dry kg	16	1		09/13/94

Reported By: James H. Johnson
Vice-President

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Report Date: 09/22/94
Date Arrived: 08/29/94
Date Sampled: 08/15/94
Time Sampled: -
Collected By: Edwin

MDL = Method Detection Limit

Our Lab #: F141796
Location/Project: School
Your Sample ID: Test Heap
Sample Matrix: Soil
Comments:

* Flag Definitions
B = Below Regulatory Min.
H = Above Regulatory Max.

Method	Parameter	Units	Results *	MDL	Date Prepared	Date Analyzed
EPA 160.3	Solids	%	87.9	1.0		09/19/94
EPA 335.2	Total Cyanide	mg/dry kg	66	23		09/07/94
SM 412H	Cyanide, Weak & Dissociable	mg/dry kg	44	8		09/13/94

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Report Date: 09/22/94

Date Arrived: 08/29/94
Date Sampled: 08/29/94
Time Sampled: -
Collected By: Edwin

MDL = Method Detection Limit

Our Lab #: F141797
Location/Project: School
Your Sample ID: Test Heap 4A
Sample Matrix: Soil
Comments:

* Flag Definitions
B = Below Regulatory Min.
H = Above Regulatory Max.

Method	Parameter	Units	Results *	MDL	Date Prepared	Date Analyzed
EPA 160.3	Solids	%	87.5	1.0		09/19/94
EPA 335.2	Total Cyanide	mg/dry kg	13	1		09/07/94
SM 412H	Cyanide, Weak & Dissociable	mg/dry kg	10	1		09/13/94

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Attn: Edwin Kreager

Report Date: 09/22/94

Date Arrived: 08/29/94
Date Sampled: 08/29/94
Time Sampled: -
Collected By: Edwin

MDL = Method Detection Limit

Our Lab #: F141798
Location/Project: School
Your Sample ID: Test Heap 48
Sample Matrix: Soil
Comments:

* Flag Definitions
B = Below Regulatory Min.
H = Above Regulatory Max.

Method	Parameter	Units	Results *	MDL	Date Prepared	Date Analyzed
EPA 160.3	Solids	%	87.0	1.0		09/19/94
EPA 335.2	Total Cyanide	mg/dry kg	74	15		09/07/94
SM 412H	Cyanide, Weak & Dissociable	mg/dry kg	11	4		09/13/94

Reported By: James H. Johnson

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Attn: Edwin Kroeger

Report Date: 09/22/94
Date Arrived: 08/29/94
Date Sampled: 08/29/94
Time Sampled: -
Collected By: Edwin

MDL = Method Detection Limit

Our Lab #: F141799
Location/Project: School
Your Sample ID: Test Heap 4C
Sample Matrix: Soil
Comments:

* Flag Definitions
B = Below Regulatory Min.
H = Above Regulatory Max.

Method	Parameter	Units	Results *	MDL	Date Prepared	Date Analyzed
EPA 160.3	Solids	%	87.6	1.0		09/19/94
EPA 335.2	Total Cyanide	mg/dry kg	32	9		09/07/94
SM 412H	Cyanide, Weak & Dissociable	mg/dry kg	6	1		09/13/94

Reported By: James H. Johnson
Vice-President

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Fairbanks AK 99775
Attn: Edwin Kroeger

Report Date: 09/22/94

Date Arrived: 08/29/94
Date Sampled: 08/29/94
Time Sampled: -
Collected By: Edwin

MDL = Method Detection Limit

Our Lab #: F141800
Location/Project: School
Your Sample ID: Test Heap 4D
Sample Matrix: Soil
Comments:

* Flag Definitions
B = Below Regulatory Min.
H = Above Regulatory Max.

Method	Parameter	Units	Results *	MDL	Date Prepared	Date Analyzed
EPA 160.3	Solids	%	87.3	1.0		09/19/94
EPA 335.2	Total Cyanide	mg/dry kg	126	7		09/07/94
SM 412H	Cyanide, Weak & Dissociable	mg/dry kg	11	8		09/13/94

Reported By: James H. Johnson
Vice-President

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Fairbanks AK 99775-5800

Attn: Peggy Arps

Report Date: 09/22/94

Date Arrived: 09/14/94
Date Sampled: 09/07/94
Time Sampled: -
Collected By: -

MDL = Method Detection Limit

Our Lab #: F142375
Location/Project: -
Your Sample ID: Pond 9/7/94
Sample Matrix: Water
Comments:

* Flag Definitions
B = Below Regulatory Min.
H = Above Regulatory Max.

Method	Parameter	Units	Results *	MDL	Date Prepared	Date Analyzed
EPA 335.2	Total Cyanide	mg/l	156	16.80		09/14/94
SM 412H	Cyanide, Weak & Dissociable	mg/l	60.7	10.00		09/19/94

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Attn: Peggy Arps

Report Date: 09/22/94

Date Arrived: 09/14/94
Date Sampled: 09/07/94
Time Sampled: -
Collected By: -

MDL = Method Detection Limit

Our Lab #: F142376
Location/Project: -
Your Sample ID: Heap Eff 9/7/94
Sample Matrix: Water
Comments:

* Flag Definitions
B = Below Regulatory Min.
H = Above Regulatory Max.

Method	Parameter	Units	Results *	MDL	Date Prepared	Date Analyzed
EPA 335.2	Total Cyanide	mg/l	630	8.40		09/14/94
SM 412H	Cyanide, Weak & Dissociable	mg/l	342	40.00		09/19/94

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Vice-President

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Attn: Peggy Arps

Report Date: 09/22/94

Date Arrived: 09/14/94
Date Sampled: 09/12/94
Time Sampled: -
Collected By: -

MDL = Method Detection Limit

Our Lab #: F142374
Location/Project: -
Your Sample ID: Heap AGG
Sample Matrix: Soil
Comments:

* Flag Definitions
B = Below Regulatory Min.
H = Above Regulatory Max.

Method	Parameter	Units	Results *	MDL	Date Prepared	Date Analyzed
EPA 160.3	Solids	%	98.6	1.0		09/19/94
EPA 335.2	Total Cyanide	mg/dry kg	28	5		09/14/94
SM 412H	Cyanide, Weak & Dissociable	mg/dry kg	35	8		09/19/94

Reported By: James H. Johnson
Vice-President

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Fairbanks AK 99775-5800

Attn: Peggy Arps

Report Date: 09/22/94

Date Arrived: 09/14/94
Date Sampled: 09/12/94
Time Sampled: -
Collected By: -

MDL = Method Detection Limit

Our Lab #: F142371
Location/Project: -
Your Sample ID: AGG 1
Sample Matrix: Soil
Comments:

* Flag Definitions
B = Below Regulatory Min.
H = Above Regulatory Max.

Method	Parameter	Units	Results *	MDL	Date Prepared	Date Analyzed
EPA 160.3	Solids	%	89.3	1.0		09/19/94
EPA 335.2	Total Cyanide	mg/dry kg	23	4		09/14/94
SM 412H	Cyanide, Weak & Dissociable	mg/dry kg	5	1		09/19/94

Reported By: James H. Johnson
Vice-President

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206 Brooks
PO Box 755800
Fairbanks AK 99775-5800

Attn: Peggy Arps

Report Date: 09/22/94

Date Arrived: 09/14/94
Date Sampled: 09/12/94
Time Sampled: -
Collected By: -

MDL = Method Detection Limit

Our Lab #: F142372
Location/Project: -
Your Sample ID: AGG 2
Sample Matrix: Soil
Comments:

* Flag Definitions
B = Below Regulatory Min.
H = Above Regulatory Max.

Method	Parameter	Units	Results *	MDL	Date Prepared	Date Analyzed
EPA 160.3	Solids	%	88.0	1.0		09/19/94
EPA 335.2	Total Cyanide	mg/dry kg	20	4		09/14/94
SM 412H	Cyanide, Weak & Dissociable	mg/dry kg	12	6		09/19/94

Reported By: James H. Johnson
Vice-President

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MIRL/UAF
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PO Box 755800
Fairbanks AK 99775-5800

Attn: Peggy Arps

Report Date: 09/22/94

Date Arrived: 09/14/94
Date Sampled: 09/12/94
Time Sampled: -
Collected By: -

MDL = Method Detection Limit

Our Lab #: F142373
Location/Project: -
Your Sample ID: AGG 3
Sample Matrix: Soil
Comments:

* Flag Definitions
B = Below Regulatory Min.
H = Above Regulatory Max.

Method	Parameter	Units	Results *	MDL	Date Prepared	Date Analyzed
EPA 160.3	Solids	%	86.1	1.0		09/19/94
EPA 335.2	Total Cyanide	mg/dry kg	38	6		09/14/94
SM 412H	Cyanide, Weak & Dissociable	mg/dry kg	6	6		09/19/94

Reported By: James H. Johnson
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Attn: Peggy Arps

Report Date: 09/22/94

Date Arrived: 09/14/94
Date Sampled: 09/13/94
Time Sampled: -
Collected By: -

MDL = Method Detection Limit

Our Lab #: F142369
Location/Project: -
Your Sample ID: Pond 9/13/94
Sample Matrix: Water
Comments:

* Flag Definitions
B = Below Regulatory Min.
H = Above Regulatory Max.

Method	Parameter	Units	Results *	MDL	Date Prepared	Date Analyzed
EPA 335.2	Total Cyanide	mg/l	424	8.40		09/14/94
SM 412H	Cyanide, Weak & Dissociable	mg/l	62	20.00		09/19/94

Reported By: James H. Johnson
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Attn: Peggy Arps

Report Date: 09/22/94

Date Arrived: 09/14/94
Date Sampled: 09/13/94
Time Sampled: -
Collected By: -

MDL = Method Detection Limit

Our Lab #: F142370
Location/Project: -
Your Sample ID: Heap Eff 9/13/94
Sample Matrix: Water
Comments:

* Flag Definitions
B = Below Regulatory Min.
H = Above Regulatory Max.

Method	Parameter	Units	Results *	MDL	Date Prepared	Date Analyzed
EPA 335.2	Total Cyanide	mg/l	436	8.40		09/14/94
SM 412H	Cyanide, Weak & Dissociable	mg/l	236	25.00		09/19/94

Reported By: James H. Johnson
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Attn: Peggy Arps

Report Date: 09/29/94

Date Arrived: 09/20/94
Date Sampled: 09/16/94
Time Sampled: -
Collected By: -

MDL = Method Detection Limit

* Flag Definitions
B = Below Regulatory Min.
H = Above Regulatory Max.

Our Lab #: F142606
Location/Project: -
Your Sample ID: Effluent Upper
Sample Matrix: Water
Comments:

Method	Parameter	Units	Results *	MDL	Date Prepared	Date Analyzed
EPA 335.2	Total Cyanide	mg/l	533	8.40		09/23/94
SM 412H	Cyanide, Weak & Dissociable	mg/l	196	80.00		09/20/94

Reported By: James H. Johnson
Vice-President

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Fairbanks AK 99775-5800

Attn: Peggy Arps

Report Date: 09/29/94

Date Arrived: 09/20/94
Date Sampled: 09/16/94
Time Sampled: -
Collected By: -

MDL = Method Detection Limit

* Flag Definitions
B = Below Regulatory Min.
H = Above Regulatory Max.

Our Lab #: F142607
Location/Project: -
Your Sample ID: Effluent Lower
Sample Matrix: Water
Comments:

Method	Parameter	Units	Results *	MDL	Date Prepared	Date Analysed
EPA 335.2	Total Cyanide	mg/l	774	8.40		09/23/94
SM 412H	Cyanide, Weak & Dissociable	mg/l	339	40.00		09/20/94

Reported By: James H. Johnson
Vice-President

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Attn: Peggy Arps

Report Date: 09/29/94

Date Arrived: 09/20/94
Date Sampled: 09/16/94
Time Sampled: -
Collected By: -

MDL = Method Detection Limit

* Flag Definitions
B = Below Regulatory Min.
H = Above Regulatory Max.

Our Lab #: F142608
Location/Project: -
Your Sample ID: Pond
Sample Matrix: Water
Comments:

Method	Parameter	Units	Results *	MDL	Date Prepared	Date Analyzed
EPA 335.2	Total Cyanide	mg/l	205	42.00		09/23/94
SM 412H	Cyanide, Weak & Dissociable	mg/l	64	40.00		09/20/94

Reported By: James H. Johnson
Vice-President

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Fairbanks AK 99775-5800

Attn: Peggy Arps

Report Date: 09/29/94

Date Arrived: 09/20/94
Date Sampled: 09/17/94
Time Sampled: -
Collected By: -

MDL = Method Detection Limit

* Flag Definitions
B = Below Regulatory Min.
H = Above Regulatory Max.

Our Lab #: F142611
Location/Project: -
Your Sample ID: Pond
Sample Matrix: Water
Comments:

Method	Parameter	Units	Results *	MDL	Date Prepared	Date Analyzed
EPA 335.2	Total Cyanide	mg/l	214	42.00		09/23/94
SM 412H	Cyanide, Weak & Dissociable	mg/l	65.2	40.00		09/20/94

Reported By: James H. Johnson
Vice-President

NORTHERN TESTING LABORATORIES, INC.

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MIRL/UAF
206 Brooks
PO Box 755800
Fairbanks AK 99775-5800

Attn: Peggy Arps

Report Date: 09/29/94

Date Arrived: 09/20/94
Date Sampled: 09/17/94
Time Sampled: -
Collected By: -

MDL = Method Detection Limit

* Flag Definitions
B = Below Regulatory Min.
H = Above Regulatory Max.

Our Lab #: F142609
Location/Project: -
Your Sample ID: Effluent Upper
Sample Matrix: Water
Comments:

Method	Parameter	Units	Results *	MDL	Date Prepared	Date Analyzed
EPA 335.2	Total Cyanide	mg/l	507	8.40		09/23/94
SM 412H	Cyanide, Weak & Dissociable	mg/l	204	40.00		09/20/94

Reported By: James H. Johnson
Vice-President

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Attn: Peggy Arps

Report Date: 09/29/94

Date Arrived: 09/20/94
Date Sampled: 09/17/94
Time Sampled: -
Collected By: -

MDL = Method Detection Limit

Our Lab #: F142610
Location/Project: -
Your Sample ID: Effluent Lower
Sample Matrix: Water
Comments:

* Flag Definitions
B = Below Regulatory Min.
H = Above Regulatory Max.

Method	Parameter	Units	Results *	MDL	Date Prepared	Date Analyzed
EPA 335.2	Total Cyanide	mg/l	865	8.40		09/23/94
SM 412H	Cyanide, Weak & Dissociable	mg/l	416	40.00		09/20/94

Reported By: James H. Johnson
Vice President

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Attn: Peggy Arps

Report Date: 09/30/94

Date Arrived: 09/20/94
Date Sampled: 09/19/94
Time Sampled: -
Collected By: -

MDL = Method Detection Limit

Our Lab #: F142614
Location/Project: -
Your Sample ID: Pond
Sample Matrix: Water
Comments:

* Flag Definitions
B = Below Regulatory Min.
H = Above Regulatory Max.

Method	Parameter	Units	Results *	MDL	Date Prepared	Date Analyzed
EPA 335.2	Total Cyanide	mg/l	180	8.40		09/27/94
SM 412H	Cyanide, Weak & Dissociable	mg/l	64	40.00		09/20/94

Reported By: James H. Johnson
Vice-President

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Attn: Peggy Arps

Report Date: 09/29/94

Date Arrived: 09/20/94
Date Sampled: 09/19/94
Time Sampled: -
Collected By: -

MDL = Method Detection Limit

Our Lab #: F142612
Location/Project: -
Your Sample ID: Effluent Upper
Sample Matrix: Water
Comments:

* Flag Definitions
B = Below Regulatory Min.
H = Above Regulatory Max.

Method	Parameter	Units	Results *	MDL	Date Prepared	Date Analyzed
EPA 335.2	Total Cyanide	mg/l	566	8.40		09/23/94
SM 412H	Cyanide, Weak & Dissociable	mg/l	203	40.00		09/20/94

Reported By: James H. Johnson
Vice-President

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Attn: Peggy Arps

Report Date: 09/29/94

Date Arrived: 09/20/94
Date Sampled: 09/19/94
Time Sampled: -
Collected By: -

MDL = Method Detection Limit

* Flag Definitions
B = Below Regulatory Min.
H = Above Regulatory Max.

Our Lab #: F142613
Location/Project: -
Your Sample ID: Effluent Lower
Sample Matrix: Water
Comments:

Method	Parameter	Units	Results *	MDL	Date Prepared	Date Analyzed
EPA 335.2	Total Cyanide	mg/l	936	8.40		09/23/94
SM 412H	Cyanide, Weak & Dissociable	mg/l	443	40.00		09/20/94

Reported By: James H. Johnson
Vice-President

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Attn: Peggy Arps

Report Date: 10/31/94

Date Arrived: 10/12/94
Date Sampled: 09/23/94
Time Sampled: -
Collected By: S Friday

MDL = Method Detection Limit

Our Lab #: F143426
Location/Project: -
Your Sample ID: Pond
Sample Matrix: Water
Comments:

* Flag Definitions
B = Below Regulatory Min.
H = Above Regulatory Max.

Method	Parameter	Units	Results *	MDL	Date Prepared	Date Analyzed
EPA 335.2	Total Cyanide	mg/l	178	33.60		10/26/94

Patricia A. Woody
Reported By: Patricia A. Woody
Senior Chemist

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Attn: Peggy Arps

Report Date: 10/31/94

Date Arrived: 10/12/94
Date Sampled: 09/23/94
Time Sampled: -
Collected By: S Friday

MDL = Method Detection Limit

Our Lab #: F143425
Location/Project: -
Your Sample ID: Upper Effluent
Sample Matrix: Water
Comments:

* Flag Definitions
B = Below Regulatory Min.
H = Above Regulatory Max.

Method	Parameter	Units	Results *	MDL	Date Prepared	Date Analyzed
EPA 335.2	Total Cyanide	mg/l	479	33.60		10/26/94

Reported By: Patricia A Woody
Senior Chemist

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Attn: Peggy Arps

Report Date: 10/31/94

Date Arrived: 10/12/94
Date Sampled: 09/23/94
Time Sampled: -
Collected By: S Friday

MDL = Method Detection Limit

Our Lab #: F143424
Location/Project: -
Your Sample ID: Lower Effluent
Sample Matrix: Water
Comments:

* Flag Definitions
B = Below Regulatory Min.
H = Above Regulatory Max.

Method	Parameter	Units	Results *	MDL	Date Prepared	Date Analyzed
EPA 335.2	Total Cyanide	mg/l	849	42.00		10/26/94

Patricia A Woody

Reported By: Patricia A. Woody
Senior Chemist

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Attn: Peggy Arps

Report Date: 10/31/94

Data Arrived: 10/12/94
Date Sampled: 09/26/94
Time Sampled: -
Collected By: S Friday

MDL = Method Detection Limit

Our Lab #: F143429
Location/Project: -
Your Sample ID: Pond
Sample Matrix: Water
Comments:

* Flag Definitions
B = Below Regulatory Min.
H = Above Regulatory Max.

Method	Parameter	Units	Results *	MDL	Date Prepared	Date Analyzed
EPA 335.2	Total Cyanide	mg/l	174	33.60		10/26/94

Patricia A. Woody

Reported By: Patricia A. Woody
Senior Chemist

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Attn: Peggy Arps

Report Date: 10/31/94
Date Arrived: 10/12/94
Date Sampled: 09/30/94
Time Sampled: -
Collected By: S Friday

MDL = Method Detection Limit

Our Lab #: F143427
Location/Project: -
Your Sample ID: Lower Effluent
Sample Matrix: Water
Comments:

* Flag Definitions
B = Below Regulatory Min.
H = Above Regulatory Max.

Method	Parameter	Units	Results *	MDL	Date Prepared	Date Analyzed
EPA 335.2	Total Cyanide	mg/l	806	33.60		10/26/94

Reported By: Patricia A. Woody
Senior Chemist

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Report Date: 10/31/94
Date Arrived: 10/12/94
Date Sampled: 09/30/94
Time Sampled: -
Collected By: S Friday

MDL = Method Detection Limit

Our Lab #: F143428
Location/Project: -
Your Sample ID: Upper Effluent
Sample Matrix: Water
Comments:

* Flag Definitions
B = Below Regulatory Min.
H = Above Regulatory Max.

Method	Parameter	Units	Results *	MDL	Date Prepared	Date Analysed
EPA 335.2	Total Cyanide	mg/l	513	33.60		10/26/94

Patricia A. Moody

Reported By: Patricia A. Moody
Senior Chemist

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Attn: Peggy Arps

Report Date: 10/31/94
Date Arrived: 10/12/94
Date Sampled: 10/06/94
Time Sampled: -
Collected By: S Friday

MDL = Method Detection Limit

Our Lab #: F143431
Location/Project: -
Your Sample ID: Upper Effluent
Sample Matrix: Water
Comments:

* Flag Definitions
B = Below Regulatory Min.
H = Above Regulatory Max.

Method	Parameter	Units	Results *	MDL	Date Prepared	Date Analyzed
EPA 335.2	Total Cyanide	mg/l	462	33.60		10/26/94

Reported By: Patricia A. Woody
Senior Chemist

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Attn: Peggy Arps

Report Date: 10/31/94

Date Arrived: 10/12/94
Date Sampled: 10/06/94
Time Sampled: -
Collected By: S Friday

MDL = Method Detection Limit

Our Lab #: F143430
Location/Project: -
Your Sample ID: Lower Effluent
Sample Matrix: Water
Comments:

* Flag Definitions
B = Below Regulatory Min.
H = Above Regulatory Max.

Method	Parameter	Units	Results *	MDL	Date Prepared	Date Analyzed
EPA 335.2	Total Cyanide	mg/l	889	42.00		10/26/94

Reported By: Patricia A. Woody
Senior Chemist

APPENDIX D: FATTY ACID IDENTIFICATION

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U.S.A.
Phone 302 737-4297

Technical Note #101
Myron Sasser
May 1990

FAX 302 737-7781

IDENTIFICATION OF BACTERIA BY GAS CHROMATOGRAPHY OF CELLULAR FATTY ACIDS

ABSTRACT

Culturing of bacteria under standardized conditions followed by extraction of the fatty acids and gas chromatographic analysis provides data for bacterial identification. Use of principal component analysis and pattern recognition software facilitates identification at the species or subspecies level. This technical note describes the MIDI automated Microbial Identification System (MIS).

INTRODUCTION

For many years, analysis of short chain fatty acids (volatile fatty acids, VFAs) has been routinely used in identification of anaerobic bacteria. In numerous scientific papers, the fatty acids between 9 and 20 carbons in length have been used to characterize genera and species of bacteria, especially those which would be categorized as nonfermentative Gram negative organisms. With the advent of fused silica capillary columns (which allow recovery of hydroxy acids and resolution of many isomers) it has become practical to use gas chromatography of whole cell fatty acid methyl esters to identify a wide range of bacteria.

FATTY ACIDS FOUND IN BACTERIA

More than 300 fatty acids and related compounds have been found in bacteria analyzed in the Microbial ID laboratory. The wealth of information contained in these compounds can be estimated by considering not only the presence or absence of each acid, but also by using the data in quantitative fashion. While the theoretical ability to differentiate among 2^{300} different combinations is not practical due to the nonrandom distribution within groups of bacteria, it is obvious that the huge number of fatty acids creates great 'naming' power within the system.

The structures of a few of these compounds are shown in Figure 1. For simplicity's sake in this note, all compounds will be referred to as fatty acids, even though the actual compounds may be aldehydes, hydrocarbons, or dimethyl acetals, and are typically analyzed as the methyl esters. The system of naming used in the note is to count carbons from the carboxyl end and to indicate the other structures where known. The various combinations of features may result in a very large numbers of fatty acids. Most fatty acid identifications have been confirmed by mass spectroscopy, but some are still listed as 'unknown' or with a letter designating that the double bond position and/or configuration has not been confirmed.

CULTURING OF THE BACTERIA

Several scientific papers have reported the effects on bacterial fatty acid composition of growth temperature and of different growth media. To minimize these variables, a temperature and growth medium have been chosen to be used for each library. For example, most aerobic bacteria will grow well on Trypticase Soy Broth Agar (TSBA) which consists of 30g Trypticase Soy Broth and 15g of agar (BBL). Aerobic bacteria which will not grow well on this medium are grown on the medium which would be most commonly used for their growth in the laboratory (eg. Legionella on buffered charcoal yeast extract, and Haemophilus on chocolate agar). The temperature chosen for the TSBA database was 28C to enable growth of a wide range of organisms. A separate database (CLIN) uses 35C and blood agar (Trypticase Soy base) as the standards, with specialized media for specific organisms. For the anaerobic bacteria, the plate-based dataset uses cultures grown at 35C on brain-heart infusion with supplements.

Fig. 1 STRUCTURE OF FATTY ACIDS

The MIS uses fatty acids 9-20 carbons in length. The peaks are automatically named and quantitated by the system. Branched chain acids predominate in some Gram positive bacteria, while short chain hydroxy acids often characterize lipopolysaccharides of the Gram negative bacteria.

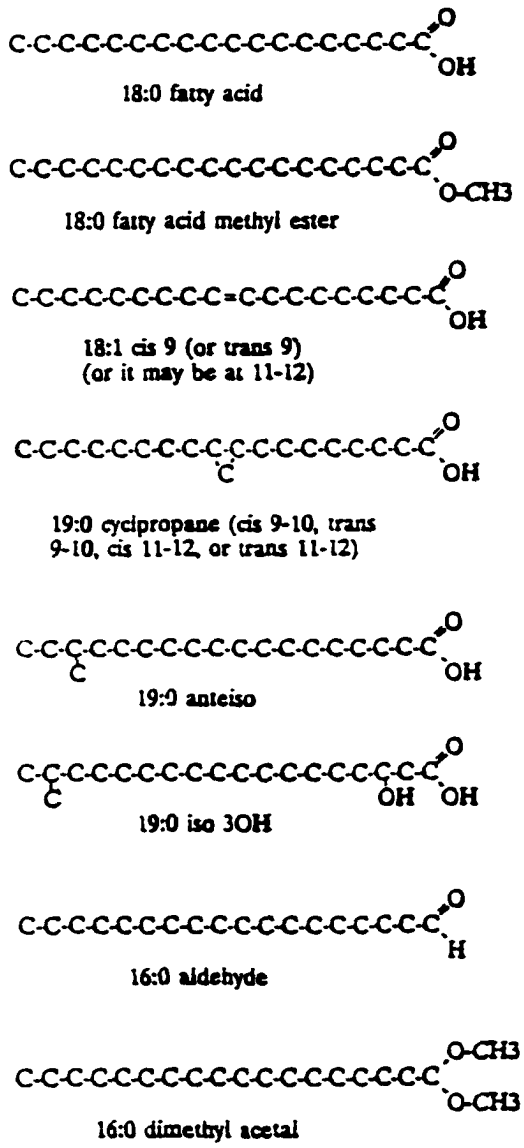
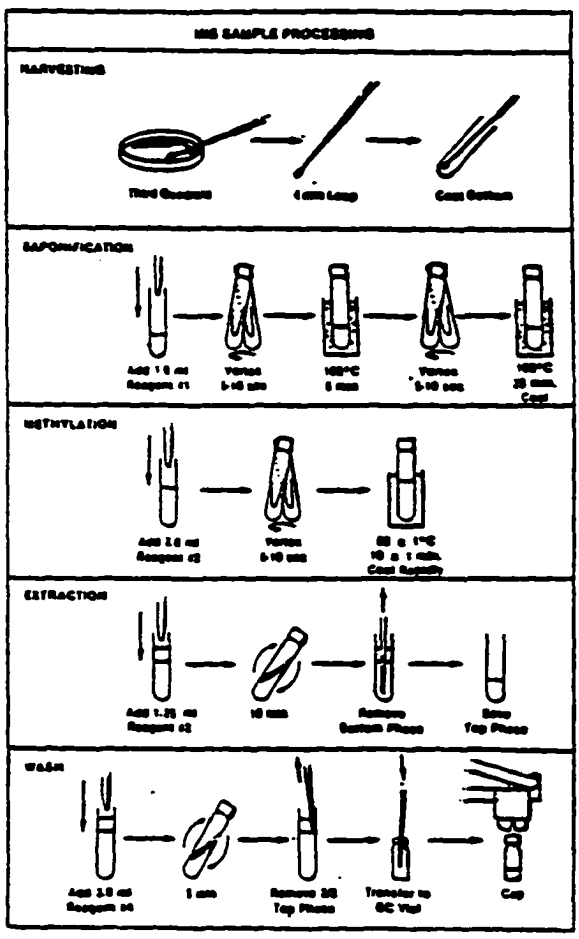


Fig. 2 FATTY ACID EXTRACTION

Following culture in broth or on a plate of medium, the bacteria are extracted in a single culture tube. Saponification, methylation, extraction into an organic solvent and washing of the extract take about 7 minutes per sample. The diagram shows an unknown aerobe being prepared for analysis and naming.



A database containing more than 250 entries was developed (by the VPI Anaerobe Lab) using overnight cultures from peptone-yeast extract-glucose broth.

The effect of age of culture is minimized in the broth cultures by harvesting at a given turbidity. When using plate cultures, (a 24 hr growth period is used in aerobes, 48 for anaerobes) and standardization of physiological age of culture is obtained by choice of sector from a quadrant streak on the plate. Slow-growing organisms may be incubated for the period of time necessary to obtain adequate growth.

REAGENTS

Four reagents are required to cleave the fatty acids from lipids (reagent 1), methylate the fatty acids (reagent 2), extract the fatty acid methyl esters into an organic solvent (reagent 3), and to clean the extract (reagent 4) so that the column is not damaged by the large numbers of runs daily handled by the MIS.

Reagent 1, Saponification - 45g sodium hydroxide, 150 ml methanol, and 150 ml distilled water. Dispensing is through use of an autopipet to assure reproducibility and to allow for large numbers of assays in a day.

Reagent 2, Methylation - 325ml of certified 6.0N hydrochloric acid and 275 ml of methyl alcohol. This drops the pH of the solution below 1.5 and causes methylation (for increased volatility in a partially polar column) of the fatty acid. The fatty acid methyl ester is poorly soluble in the aqueous phase at this point.

Reagent 3, Extraction - 200ml of hexane and 200 ml of methyl tert-butyl ether. These will extract the fatty acid methyl esters into the organic phase for use with the gas chromatograph.

Reagent 4, Sample Cleanup - 10.8g of sodium hydroxide dissolved in 900ml of distilled water. This procedure reduces contamination of the injection port liner, the column, and of the detector. More than 10,000 analyses have been performed on a column prior to needing any maintenance.

SAMPLE PROCESSING

The five steps to prepare GC ready extracts are illustrated in Figure 2, and are summarized in the following:

Harvesting - A 4mm loop is used to harvest about 40mg of bacterial cells from the third quadrant (second or first quadrant if slow growing) of the quadrant streaked plate. The cells are placed in clean 13x100 culture tubes.

Saponification - 1.0 ml of Reagent 1 is added to each of the tubes containing cells. The tubes are securely sealed with teflon lined cap and heated in a boiling water bath for ca. 5 minutes, at which time the tube is vigorously vortexed for 5-10 seconds and returned to the water bath to complete the 30 minute heating.

Methylation - The cooled tubes are uncapped and 2 ml of Reagent 2 are added. After recapping, the tubes are heated for 10±1 minutes at 80±1C. (This step is critical in time and temperature.)

Extraction - Addition of 1.25 ml of Reagent 3 to the cooled tube is followed by recapping and gentle tumbling on a clinical rotator for about 10 minutes. The tube is uncapped and the aqueous (lower) phase is pipetted out and discarded.

Base wash - About 3 ml of Reagent 4 is added to the organic phase remaining in the tube, the tube recapped, and tumbled for 5 min. Following uncapping, about 2/3 of the organic phase is pipetted into a GC vial which is then capped and is ready for analysis.

HARDWARE FACTORS

Column - A 25m x 0.2 mm phenyl methy silicone fused silica capillary column has both the chromatographic performance and the column lifetime desired for routine analysis of bacterial extracts. The column is required to have more than 4,000 theoretical plates per meter for peaks with a k= 7 to 9. Since the stationary phase is cross linked to the fused silica tube, there is less noise, and drift during temperature programmed runs.

Gas chromatograph - The temperature program ramps from 170C to 270C at 5C per minute. Following the analysis, a ballistic increase to 300C allows cleaning of the column during a hold of 2 minutes. The flame ionization detector allows for a large dynamic range and provides good sensitivity. Hydrogen is the carrier gas, nitrogen is the 'makeup' gas, and air is used to support the flame.

Autosampler - Use of an autosampler allows the system to be operated unattended for up to 2 days at a time. Samples are logged into the computerized sample table and all sampling (including STAT samples) are run automatically.

Figure 3. Overview of extraction, analysis and naming of an unknown culture.

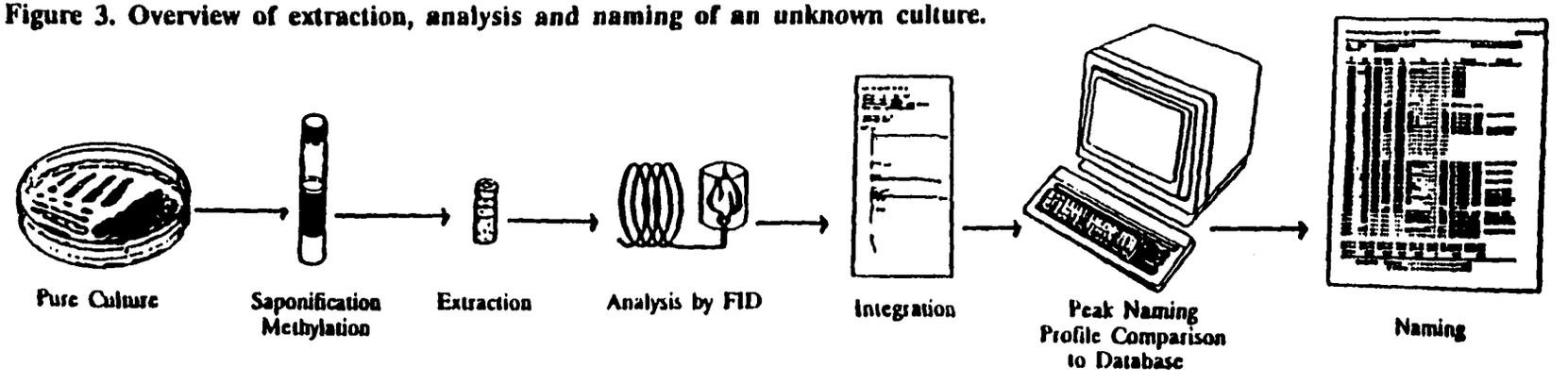
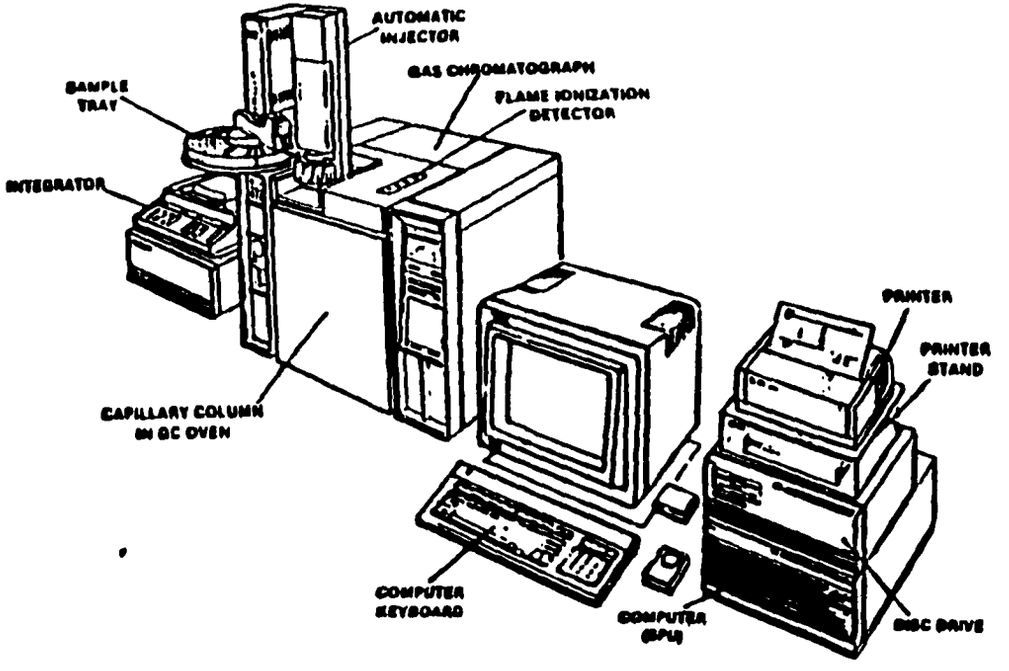


Figure 4. Diagram of the hardware of the Microbial Identification System.



Integrator and Computer - The electronic signal is passed from the GC detector to the integrator and after processing is sent to the computer. Storage of the data on the hard disc is automatic, and the data may also be automatically compared to 'libraries' of data.

CALIBRATION AND PEAK NAMING

The Microbial Identification System uses an external calibration mixture (available from MIDI or from Hewlett Packard). The standard is a mixture of the straight chain saturated fatty acids from 9 to 20 carbons in length (9:0 to 20:0) and has five hydroxy acids in the mixture. All compounds are added quantitatively so that the gas chromatographic performance may be evaluated by the software each time the calibration mixture is analyzed. The hydroxy compounds are especially sensitive to changes in pressure/temperature relationships and to contamination of the injection port liner, and thus are quality control checks for these matters.

Retention time data obtained from injecting the calibration mixture may be converted to Equivalent Chain Length (ECL) data for bacterial fatty acid naming. The ECL value for each fatty acid can be derived as a function of its elution time in relation to the elution times of a known series of straight chain saturated fatty acids.

$$ECL_x = \frac{R_{tx} - R_{tn}}{R_{t(n+1)} - R_{tn}} + n$$

Where R_{tx} is the retention time of x; R_{tn} is the retention time of the saturated fatty acid methyl ester preceding x; $R_{t(n+1)}$ is the retention time of the saturated fatty acid methyl ester eluting after x.

Thus it is possible, by comparison to the external standard, to compute the ECL value for each compound following analysis. The GC and column currently used allow windows to be set at 0.010 ECL unit wide giving great precision in resolution of isomers. After naming the peaks in an unknown sample, the MIS compares the ECL values for the most stable series (eg. saturated straight chain or branched chain acids) to the peak naming table theoretically perfect values and may recalibrate (internally) if sufficient differences are detected. This feature allows the system to be run for up to two days unattended without worrying about drift between runs.

LIBRARIES

The databases consist of more than 60,000 analyses of strains obtained from experts and from culture collections. The cultures were collected from around the world to avoid potential geographic bias. Where possible, 20 or more strains of a species or subspecies were analyzed to make the entry. When chromatographic subgroups were found within a taxon, more strains were obtained to deliniate each group.

The method of culture (thus the corresponding library) is indicated in the name field of the sample as it is logged into the computer. Analysis of an unknown results in an automatic comparison of the composition of the unknown strain to a stored database using a covariance matrix, principal component analysis and pattern recognition software. The covariance matrix takes into account the mole-for-mole relationship of conversion of one fatty acid to another (eg. 16:0 to 16:1 due to action of a desaturase) which might occur in relation to a temperature shift, or in age differences. The pattern recognition software uses calculations of cross terms (eg. ratios between fatty acid amounts) in addition to the principal component base. The subtle differences between biovars or subspecies depends upon the power of the pattern recognition software to discriminate at this level.

Because the libraries are open ended (not limited by a finite set of biochemical assays), the number of species in them is large and growing. Also since it is as easy to extract and analyze any one strain as it is any other, the libraries are only limited by the ability of MIDI to obtain adequate numbers of strains to make the entries. Of course, some groups of bacteria are more amenable to fatty acid composition use for identification than are others, and these seem to relate to whether well characterized strains are available and to whether 'species' can be justified on the basis of DNA relatedness (eg. Escherichia coli is related at the species level to Shigella dysenteriae, (see Brenner, 1973).

SUMMARY

The Microbial Identification System is a fully automated gas chromatographic analytical system which identifies bacteria based on their unique fatty acid profiles. The system will analyze about 45 samples per day, at a cost of about $1.30 per sample in consumables. Since a technician can extract 75 samples per day, the operator time per sample averages about 6 minutes. Because no subjective tests are required, the naming is highly objective and reproducible.

Figure 5. Chromatographic run of a common aerobic culture.

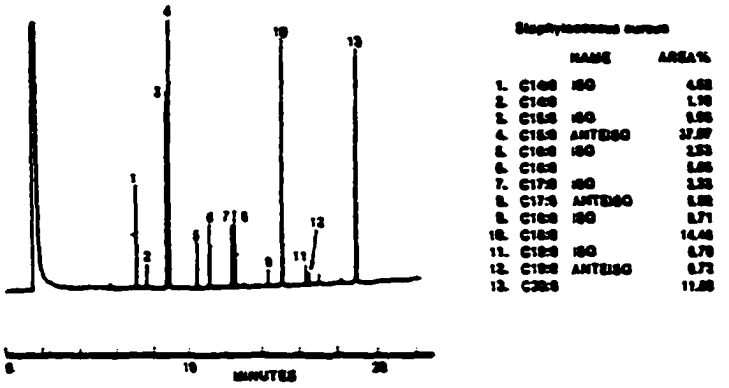


Table 1. Estimated cost of all consumables for plate-grown culture.

CONSUMABLES	Source	Part Number	Est. $ Price	Amount	$ Cost/ Sample	Number Samples
TSBA AEROBIC CULTURE TECHNIQUE						
Granular Agar	BBL	11849	75	1 lb	0.02	3,750
Trypticase soy broth (TSB)	BBL	11768	19	1 lb	0.01	1,875
Culture dishes, disposable 100x15	Thomas	3488-828	54	500	0.11	500
SAMPLE PREPARATION						
Sodium hydroxide, ACS grade, pellets	Baker	3722	60	2.5 kg	0.01	13,000
Methanol (methyl alcohol) HPLC grade	Baker	9093	31	4 L	0.01	700
Hydrochloric acid 6N	Baxter	H168-1NY	10	1 L	0.01	900
Hexane, HPLC grade	Baker	9304	91	4 L	0.06	1,500
Methyl-tert butyl ether (MTBE)	Aldrich	29.321-0	43	3 L	0.04	1,125
Crimp top sampler vials, 2 ml	H-P	5180-4197	20	100	0.20	100
Crimp caps, 11 mm orange	H-P	5181-1210	12	100	0.12	100
Crimp caps, 11 mm red, for stds.	H-P	5181-1211	38	100	0.03	1,400
Pasteur pipets, 9 inch disposaole	Thomas	7760-B62	63	1000	0.06	1,000
GAS CHROMATOGRAPHIC SYSTEM						
HP 7673A Sampler Syringe	H-P	9301-0713	35	1	0.01	5,000
Injection port septa, 11 mm grey	H-P	5080-8894	55	144	0.01	15,000
Inj. port liner, straight glass	H-P	19251-60540	35	1	0.02	2,000
Silanized glass wool for inj. port liner	Supelco	2-0411	15	50 gm	0.01	15,000
Viton O-ring for nigh temperature	H-P	5180-4182	6	10	0.01	5,000
Graphite column ferrule, 0.5 mm ID	H-P	5080-8853	25	10	0.01	5,000
MIS calibration standards kit	MIDI	1200	125	1 set	0.18	700
3396A Integrator paper	H-P	5181-1219	59	4 rolls	0.04	1,600
Capillary column	H-P	190918-102	450	1	0.05	10,000
Hydrogen gas, 99.995% pure	Local		119	6	0.10	7,000
Nitrogen gas, 99.995% pure	Local		104	2	0.03	7,000
Air, best quality available	Local		27	24	0.09	7,000
COMPUTER SYSTEM						
HP 2225A Printer paper, sheets	H-P	92261N	50	2500	0.02	2,500
HP 2225A Printer print head	H-P	92261A	8	1	0.01	1,000
Double sided flexible discs (FLOPPY)	H-P	92192A	29	10	0.01	5,000

Estimated Consumables Cost per Analysis (including gases).....$1.30

Prices subject to change. Prices given for budgeting purposes.

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APPENDIX E: MIDI RESULTS

MIDI DOS SYSTEM Version: 4.15 FLOPPY:F92221623 16-OCT-95 11:36:42

ID: 327 UM-U.S.-BUREAU-MINES-2 (GS-OP KD Date of run: 22-FEB-92 00:59:43
Bottle: 16 SAMPLE [AEROBE]

RT	Area	Ar/Ht	Respon	ECL	Name	%	Comment 1		Comment 2
1.665	787082752	0.038	. . .	6.996	SOLVENT PEAK		< min rt		
1.885	6592	0.027	. . .	7.440	. . . . .		< min rt		
2.100	4136	0.026	. . .	7.873	. . . . .		< min rt		
2.633	456	0.029	. . .	8.947	. . . . .		< min rt		
4.220	4376	0.030	1.067	11.426	10:0 3OH	3.68	ECL deviates	0.003	
4.740	11416	0.032	1.045	12.000	12:0	9.41	ECL deviates	0.000	Reference -0.006
6.484	5424	0.036	1.000	13.456	12:0 3OH	4.28	ECL deviates	0.001	
7.217	632	0.038	0.986	14.000	14:0	0.49	ECL deviates	-0.000	Reference -0.004
8.733	1256	0.040	0.967	15.001	15:0	0.96	ECL deviates	0.001	Reference -0.003
10.073	21736	0.041	0.955	15.817	Sum In Feature 4 . .	16.36	ECL deviates	0.000	16:1 w7c/15 iso 2OH
10.131	8568	0.039	0.954	15.853	Sum In Feature 4 . .	6.45	ECL deviates	0.006	15:0 ISO 2OH/16:1w7c
10.370	15432	0.041	0.952	15.998	16:0	11.59	ECL deviates	-0.002	Reference -0.005
11.727	2480	0.044	0.944	16.795	17:1 w8c	1.85	ECL deviates	0.003	
12.080	1504	0.046	0.942	17.002	17:0	1.12	ECL deviates	0.002	Reference -0.001
13.508	58320	0.045	0.937	17.824	Sum In Feature 7 . .	43.11	ECL deviates	-0.001	18:1 w9c/w12t/w7c
13.812	960	0.044	0.936	17.999	18:0	0.71	ECL deviates	-0.001	Reference -0.005
*******	30304	. . .	. . .	. . .	SUMMED FEATURE 4 . .	22.81	16:1 w7c/15 iso 2OH		15:0 ISO 2OH/16:1w7c
*******	58320	. . .	. . .	. . .	SUMMED FEATURE 7 . .	43.11	18:1 w7c/w9t/w12t		18:1 w9c/w12t/w7c
*******	. . . . .	. . .	. . .	. . .	. . . . .		18:1 w12t/w9t/w7c		

Solvent Ar	Total Area	Named Area	% Named	Total Amnt	Nbr Ref	ECL Deviation	Ref ECL Shift
787082752	132104	132104	100.00	126791	6	0.002	0.004

TSBA [Rev 3.90] Pseudononas 0.854
P. pseudoalcaligenes 0.854
P. alcaligenes 0.460

MIDI DOS SYSTEM Version: 4.15 FLOPPY:F92224361 16-OCT-95 11:36:42

ID: 356 UN-U.S.-BUREAU-MINES-2 (Cl-I-DIL KD Date of run: 24-FEB-92 11:09:28
Bottle: 45 STAT SAMPLE [AEROBE]

RT	Area	Ar/Ht	Respon	ECL	Name	%	Comment 1	Comment 2
1.667	754771456	0.038	. . .	6.982	SOLVENT PEAK	. . .	< min rt	
1.889	5928	0.027	. . .	7.431	. . . . .	. . .	< min rt	
2.104	3888	0.025	. . .	7.866	. . . . .	. . .	< min rt	
2.636	776	0.025	. . .	8.945	. . . . .	. . .	< min rt	
3.159	472	0.029	1.133	10.000	10:0	0.13	ECL deviates -0.000	Reference 0.001
3.842	744	0.032	1.084	11.000	11:0	0.20	ECL deviates 0.000	Reference 0.002
4.223	12368	0.029	1.066	11.422	10:0 3OH	3.30	ECL deviates -0.001	
4.563	1096	0.030	1.051	11.798	unknown 11.798 . . .	0.29	ECL deviates 0.000	
4.745	28000	0.031	1.044	11.999	12:0	7.33	ECL deviates -0.001	Reference 0.002
5.240	1352	0.032	1.029	12.438	11:0 3OH	0.35	ECL deviates -0.003	
5.438	568	0.035	1.023	12.613	13:0 ISO	0.15	ECL deviates 0.001	Reference 0.004
5.875	1456	0.034	1.012	13.000	13:0	0.37	ECL deviates 0.000	Reference 0.002
6.485	13192	0.035	1.000	13.453	12:0 3OH	3.31	ECL deviates -0.002	
6.970	1152	0.039	. . .	13.813	. . . . .	. . .		
7.219	1272	0.038	0.987	13.998	14:0	0.31	ECL deviates -0.002	Reference 0.000
8.516	1744	0.044	0.971	14.856	15:1 w6c	0.42	ECL deviates 0.000	
8.734	4808	0.039	0.968	15.001	15:0	1.17	ECL deviates 0.001	Reference 0.001
10.074	79016	0.041	0.957	15.818	Sum In Feature 4 . .	18.95	ECL deviates 0.001	16:1 w7c/15 iso 2OH
10.131	18664	0.038	0.956	15.852	Sum In Feature 4 . .	4.47	ECL deviates 0.005	15:0 ISO 2OH/16:1w7c
10.370	45216	0.040	0.954	15.998	16:0	10.82	ECL deviates -0.002	Reference -0.002
11.446	3168	0.083	0.948	16.630	17:0 ISO	0.75	ECL deviates 0.001	Reference -0.000
11.725	11496	0.044	0.946	16.794	17:1 w8c	2.73	ECL deviates 0.002	
11.845	5520	0.054	0.946	16.865	17:1 w6c	1.31	ECL deviates 0.003	
12.077	4816	0.044	0.945	17.001	17:0	1.14	ECL deviates 0.001	Reference -0.001
13.330	688	0.043	0.939	17.723	Sum In Feature 6 . .	0.16	ECL deviates 0.003	18:2 w6,9c/18:0 ANTE
13.510	176048	0.045	0.939	17.826	Sum In Feature 7 . .	41.43	ECL deviates 0.001	18:1 w9c/w12t/w7c
13.810	2112	0.045	0.938	17.999	18:0	0.50	ECL deviates -0.001	Reference -0.005
14.835	2856	0.045	. . .	18.594	. . . . .	. . .		
15.051	1736	0.045	0.934	18.719	19:0 ANTEISO	0.41	ECL deviates -0.010	
15.200	2416	0.059	. . .	18.806	. . . . .	. . .		
16.754	2872	0.047	. . .	19.714	. . . . .	. . .		
18.230	2704	0.050	. . .	20.571	. . . . .	. . .	> max rt	
18.420	9816	0.084	. . .	20.682	. . . . .	. . .	> max rt	
*******	97680	. . . . .		. . .	SUMMED FEATURE 4 . .	23.42	16:1 w7c/15 iso 2OH	15:0 ISO 2OH/16:1w7c
*******	688	. . . . .		. . .	SUMMED FEATURE 6 . .	0.16	18:2 w6,9c/18:0 ANTE	18:0 ANTE/18:2 w6,9c
*******	176048	. . . . .		. . .	SUMMED FEATURE 7 . .	41.43	18:1 w7c/w9t/w12t	18:1 w9c/w12t/w7c
*******	. . . . .	. . . . .		. . .	. . . . .	. . .	18:1 w12t/w9t/w7c	

Solvent Ar	Total Area	Named Area	% Named	Total Amnt	Nbr Ref	ECL Deviation	Ref ECL Shift
754771456	424848	415552	97.81	398868	11	0.003	0.002

TSBA [Rev 3.90] Pseudomonas 0.777
P. pseudoalcaligenes . . . . . 0.777 ✓ KD
P. alcaligenes 0.599

MIDI DOS SYSTEM Version: 4.15 FLOPPY:F92221623 16-OCT-95 11:36:42

ID: 328 UW-U.S.-BUREAU-MINES-2 (GS-OR KD Date of run: 22-FEB-92 01:29:42
Bottle: 17 SAMPLE [AEROBE]

RT	Area	Ar/Ht	Respon	ECL	Name	%	Comment 1	Comment 2
1.665	784025600	0.038	. . .	6.990	SOLVENT PEAK	. . .	< min rt	
1.885	6536	0.027	. . .	7.434	. . . . .	. . .	< min rt	
2.100	4264	0.027	. . .	7.867	. . . . .	. . .	< min rt	
3.571	1120	0.030	1.103	10.606	11:0 ISO	0.86	ECL deviates 0.001	Reference -0.000
4.389	1576	0.032	1.060	11.608	12:0 ISO	1.17	ECL deviates -0.000	Reference -0.002
4.742	1872	0.032	1.045	11.998	12:0	1.37	ECL deviates -0.002	Reference -0.003
5.436	25928	0.032	1.024	12.613	13:0 ISO	18.55	ECL deviates 0.001	Reference -0.001
5.536	17600	0.033	1.021	12.701	13:0 ANTEISO	12.56	ECL deviates 0.000	Reference -0.002
6.705	568	0.037	0.995	13.618	14:0 ISO	0.40	ECL deviates -0.000	Reference -0.002
7.217	3680	0.037	0.986	13.998	14:0	2.54	ECL deviates -0.002	Reference -0.004
8.162	8424	0.038	0.974	14.622	15:0 ISO	5.73	ECL deviates 0.001	Reference -0.001
8.299	1784	0.041	0.972	14.713	15:0 ANTEISO	1.21	ECL deviates 0.002	Reference -0.000
9.636	3704	0.042	0.958	15.550	16:0 N alcohol . . .	2.48	ECL deviates 0.001	
9.760	736	0.043	0.957	15.626	16:0 ISO	0.49	ECL deviates -0.000	Reference -0.003
9.978	4440	0.041	0.955	15.759	16:1 w11c	2.96	ECL deviates 0.002	
10.370	45512	0.041	0.952	15.998	16:0	30.28	ECL deviates -0.002	Reference -0.005
10.708	752	0.049	. . .	16.196	. . . . .	. . .		
11.447	11152	0.042	0.946	16.630	17:0 ISO	7.37	ECL deviates 0.001	Reference -0.002
11.605	1312	0.043	0.945	16.723	17:0 ANTEISO	0.87	ECL deviates 0.001	Reference -0.002
12.079	1224	0.045	0.942	17.001	17:0	0.81	ECL deviates 0.001	Reference -0.002
13.084	1200	0.046	0.939	17.580	18:3 w6c (6,9,12) .	0.79	ECL deviates 0.003	
13.405	1464	0.051	0.937	17.764	18:1 w9c	0.96	ECL deviates -0.005	
13.812	12240	0.045	0.936	17.999	18:0	8.01	ECL deviates -0.001	Reference -0.005
16.230	952	0.052	0.933	19.402	20:4 w6,9,12,15c . .	0.62	ECL deviates 0.007	

Solvent Ar	Total Area	Named Area	% Named	Total Amnt	Nbr Ref	ECL Deviation	Ref ECL Shift
784025600	147240	146488	99.49	143137	15	0.002	0.003

TSBA [Rev 3.90] Brevibacterium . . . . . 0.249 (not the type strain)
B. acetylicum . . . . . 0.249 (not the type strain)
B. a. GC subgroup B . . . . . 0.249 (not the type strain)

weak species naming

MIDI DOS SYSTEM Version: 1.15 FLOPPY:F92221623 16-OCT-95 11:36:42

ID: 329 UW-U.S.-BUREAU-MINES-2 (GS-W KD Date of run: 22-FEB-92 01:59:41
Bottle: 18 SAMPLE [AEROBE]

RT	Area	Ar/Ht	Respon	ECL	Name	%	Comment 1	Comment 2
1.666	787101696	0.038	. . .	6.994	SOLVENT PEAK	. . .	< min rt	
1.886	6568	0.027	. . .	7.437	. . . . .	. . .	< min rt	
2.101	4296	0.026	. . .	7.871	. . . . .	. . .	< min rt	
6.487	1504	0.038	1.000	13.458	12:0 3OH	3.16	ECL deviates 0.003	
7.219	1024	0.039	0.986	14.001	14:0	2.12	ECL deviates 0.001	Reference -0.002
10.075	2320	0.042	0.955	15.817	Sum In Feature 4 . .	4.65	ECL deviates 0.000	16:1 w7c/15 iso 2OH
10.371	15512	0.040	0.952	15.998	16:0	31.01	ECL deviates -0.002	Reference -0.004
13.509	27952	0.044	0.937	17.821	Sum In Feature 7 . .	54.99	ECL deviates -0.001	18:1 w7c/w9t/w12t
13.640	1168	0.058	. . .	17.897	. . . . .	. . .		
15.378	2080	0.052	0.933	18.901	19:0 CYCLO w8c . . .	4.08	ECL deviates 0.001	Reference 0.002
*******	2320	. . .	. . .	. . .	SUMMED FEATURE 4 . .	4.65	16:1 w7c/15 iso 2OH	15:0 ISO 2OH/16:1w7c
*******	27952	. . .	. . .	. . .	SUMMED FEATURE 7 . .	54.99	18:1 w7c/w9t/w12t	18:1 w9c/w12t/w7c
*******	. . . . .	. . .	. . .	. . .	. . . . .	. . .	18:1 w12t/w9t/w7c	

Solvent Ar	Total Area	Named Area	% Named	Total Amnt	Nbr Ref	ECL Deviation	Ref ECL Shift
787101696	51560	50392	97.73	47638	3	0.002	0.003

TSBA [Rev 3.90] Brevundimonas . . . . . 0.548 (Pseudomonas diminuta)
B. diminuta* . . . . . 0.548 (Pseudomonas diminuta) ✓kd
B. vesicularis* . . . . . 0.321 (Pseudomonas vesicularis)

APPENDIX F: COST SPREADSHEETS

MOST LIKELY COST SCENARIO

	INSITU TECHNIQUES			EXSITU TECHNIQUES				
	Biological	Peroxide	Chlorination	Biological	INCO	Peroxide	Chlorination	
Total Detoxification Cost	$413,105	$1,049,362	$1,151,389	$919,311	$1,294,467	$1,325,246	$1,354,434	
Operating Parameters:								
Rinse solution pumping rate	1000	gallons/min						
Hours of pumping per pore volume of rinsate	623	hours						
Daily pumping time	8	hours/day						
Days of pumping per pore volume	78	days						
Final WAD cyanide concentration in leachate	< 0.2	mg/liter						
Cyanide Concentration:								
Starting cyanide concentration in wastewater	250	250	250	250	250	250	250	mg/liter
Cyanide concentration after 1 pore volume	194.7	204.7	215.2	223.7	223.7	223.7	223.7	mg/liter
Cyanide concentration after 2 pore volumes	33.8	50.5	75.3	104.8	104.8	104.8	104.8	mg/liter
Cyanide concentration after 3 pore volumes	0.29	1.13	4.36	13.8	13.8	13.8	13.8	mg/liter
Cyanide concentration after 4 pore volumes	2.8E-05	6.9E-04	0.017	0.27	0.27	0.27	0.27	mg/liter
Cyanide concentration after 5 pore volumes				4.5E-04	4.5E-04	4.5E-04	4.5E-04	mg/liter
Capital Costs	$89,366	$74,866	$74,866	$407,316	$76,042	$74,866	$74,866	

	INSITU TECHNIQUES			EXSITU TECHNIQUES				
Operating Costs:	Biological	Peroxide	Chlorination	Biological	INCO	Peroxide	Chlorination	
Personnel	$123,464	$96,464	$96,464	$123,464	$96,464	$96,464	$96,464	$/year
INCO Royalty					0.13			$/pound
Oxidant/cyanide ratio		7	8		8	7	8	lb/lb
Alkaline/cyanide ratio			6		8		6	lb/lb
Chemical Reagents								
Hypochlorite			$0.55				$0.55	$/pound
H202		$0.90				$0.90		$/pound
SO2					$0.30			$/pound
Alkaline			$0.35		$0.35		$0.35	$/pound
Phosphate Cost	$0.95			$0.95				$/pound
Concentration	25			25				ppm
Growth Media Cost	$1.55			$0.131				$/gallon
Strength	10%			100%				
Initial Volume	80,000			100,000				gallons
Yearly Volume	10,000							gallons
Electricity	$0.13032	$0.13032	$0.13032	$0.13032	$0.13032	$0.13032	$0.13032	$/kwhr

Year 1							
Pore Volume 1							
INCO Royalty					$64,592		
Oxidant		$447,074	$319,449		$177,450	$465,807	$325,325
Alkaline			$152,464		$207,025		$155,269
Phosphate	$7,414			$7,414			
Growth media	$12,400			$13,100			
Electricity for sprinkler pumps	$3,030	$3,030	$3,030	$3,030	$3,030	$3,030	$3,030
Electricity for aeration pump					$182		
Electricity for mixing pumps	$8	$8	$8	$8	$8	$8	$8
Electricity for heap drainage pump	$3,030	$3,030	$3,030	$3,030	$3,030	$3,030	$3,030
Pore Volume 2							
INCO Royalty					$44,790		
Oxidant		$250,887	$199,477		$123,049	$323,004	$225,590
Alkaline			$95,205		$143,557		$107,668
Phosphate	$7,414			$7,414			
Growth media	$0						
Electricity for sprinkler pumps	$3,030	$3,030	$3,030	$3,030	$3,030	$3,030	$3,030
Electricity for aeration pump					$182		
Electricity for mixing pumps	$8	$8	$8	$8	$8	$8	$8
Electricity for heap drainage pump	$3,030	$3,030	$3,030	$3,030	$3,030	$3,030	$3,030
Manpower	$123,464	$96,464	$96,464	$123,464	$96,464	$96,464	$96,464
Maintenance (5% of capital cost)	$4,468	$3,743	$3,743	$20,366	$3,802	$3,743	$3,743
Total for Year 1	**$167,295**	**$810,303**	**$878,937**	**$183,892**	**$873,229**	**$901,153**	**$926,195**

Year 2							
Pore Volume 3							
INCO Royalty					$16,160		
Oxidant		$50,740	$54,700		$44,397	$116,541	$81,394
Alkaline			$26,107		$51,796		$38,847
Phosphate	$7,414			$7,414			
Growth media	$1,550						
Electricity for sprinkler pumps	$3,030	$3,030	$3,030	$3,030	$3,030	$3,030	$3,030
Electricity for aeration pump					$182		
Electricity for mixing pumps	$8	$8	$8	$8	$8	$8	$8
Electricity for heap drainage pump	$3,030	$3,030	$3,030	$3,030	$3,030	$3,030	$3,030
Pore Volume 4							
INCO Royalty					$1,913		
Oxidant		$1,111	$3,003		$5,256	$13,798	$9,636

Alkaline			$1,433		$6,132		$4,599
Phosphate	$7,414			$7,414			
Growth media							
Electricity for sprinkler pumps	$3,030	$3,030	$3,030	$3,030	$3,030	$3,030	$3,030
Electricity for aeration pump					$182		
Electricity for mixing pumps	$8	$8	$8	$8	$8	$8	$8
Electricity for heap drainage pump	$3,030	$3,030	$3,030	$3,030	$3,030	$3,030	$3,030
Manpower	$123,464	$96,464	$96,464	$123,464	$96,464	$96,464	$96,464
Maintenance (5% of capital cost)	$4,468	$3,743	$3,743	$20,366	$3,802	$3,743	$3,743
Total for Year 2	**$156,445**	**$164,193**	**$197,586**	**$170,792**	**$238,420**	**$242,681**	**$246,819**
Year 3							
Pore Volume 5							
INCO Royalty					$37		
Oxidant					$103	$270	$189
Alkaline					$120		$90
Phosphate				$7,414			
Growth media							
Electricity for sprinkler pumps				$3,030	$3,030	$3,030	$3,030
Electricity for aeration pump					$182		
Electricity for mixing pumps				$8	$8	$8	$8
Electricity for heap drainage pump				$3,030	$3,030	$3,030	$3,030
Manpower				$123,464	$96,464	$96,464	$96,464
Maintenance (5% of capital cost)				$20,366	$3,802	$3,743	$3,743
Total for Year 3				**$157,311**	**$106,776**	**$106,545**	**$106,554**

APPENDIX G: OUTPUT FROM SEE

In situ Biological Method

Cash Flows

PWC = $413,106.63

Year	Time 0	1	2
-Labor		-123,464	-123,464
-Phosphate		-14,827	-14,827
-Maintenance		-4,468	-4,468
-Electricity		-12,136	-12,136
-Growth Medium		-12,400	-1,550
-Total Oper Costs		-167,295	-156,445
-Capital Costs		-89,366	
Cash Flow		-256,661	-156,445

Sensitivity Analysis

Case Number	Capital Costs	Net Present Value	ROR
1	25.00	-346,082	0.00
2	50.00	-368,424	0.00
3	75.00	-390,765	0.00
4	100.00	-413,107	0.00
5	125.00	-435,448	0.00
6	150.00	-457,790	0.00
7	175.00	-480,131	0.00
8	200.00	-502,473	0.00

Case Number	Electricit	Net Present Value	ROR
1	25.00	-394,903	0.00
2	50.00	-400,971	0.00
3	75.00	-407,039	0.00
4	100.00	-413,107	0.00
5	125.00	-419,175	0.00
6	150.00	-425,243	0.00
7	175.00	-431,311	0.00
8	200.00	-437,379	0.00

Case Number	Growth Medium Cost	Net Present Value	ROR
1	25.00	-402,644	0.00
2	50.00	-406,132	0.00
3	75.00	-409,619	0.00
4	100.00	-413,107	0.00
5	125.00	-416,594	0.00
6	150.00	-420,082	0.00
7	175.00	-423,569	0.00
8	200.00	-427,057	0.00

Case Number	Growth Medium Stren.	Net Present Value	ROR
1	25.00	-402,644	0.00
2	50.00	-406,132	0.00
3	75.00	-409,619	0.00
4	100.00	-413,107	0.00
5	125.00	-416,594	0.00
6	150.00	-420,082	0.00
7	175.00	-423,569	0.00
8	200.00	-427,057	0.00

Case Number	Inoculum Volume	Net Present Value	ROR
1	25.00	-402,644	0.00
2	50.00	-406,132	0.00
3	75.00	-409,619	0.00
4	100.00	-413,107	0.00
5	125.00	-416,594	0.00
6	150.00	-420,082	0.00
7	175.00	-423,569	0.00
8	200.00	-427,057	0.00

Case Number	Labor	Net Present Value	ROR
1	25.00	-227,911	0.00
2	50.00	-289,643	0.00
3	75.00	-351,375	0.00
4	100.00	-413,107	0.00
5	125.00	-474,839	0.00
6	150.00	-536,571	0.00
7	175.00	-598,303	0.00
8	200.00	-660,035	0.00

Case Number	Maintenanc	Net Present Value	ROR
1	25.00	-406,404	0.00
2	50.00	-408,638	0.00
3	75.00	-410,872	0.00
4	100.00	-413,107	0.00
5	125.00	-415,341	0.00
6	150.00	-417,575	0.00
7	175.00	-419,809	0.00
8	200.00	-422,043	0.00

Case Number	Phosphate Cost	Net Present Value	ROR
1	25.00	-390,866	0.00
2	50.00	-398,280	0.00
3	75.00	-405,693	0.00
4	100.00	-413,107	0.00
5	125.00	-420,520	0.00
6	150.00	-427,934	0.00
7	175.00	-435,347	0.00
8	200.00	-442,761	0.00

Case Number	Phosphate Conc.	Net Present Value	ROR
1	25.00	-390,866	0.00
2	50.00	-398,280	0.00
3	75.00	-405,693	0.00
4	100.00	-413,107	0.00
5	125.00	-420,520	0.00
6	150.00	-427,934	0.00
7	175.00	-435,347	0.00
8	200.00	-442,761	0.00

In situ Peroxide Method

Cash Flows

PWC =1,049,362.46

Year	Time 0	1	2
-Labor		-96,464	-96,464
-Hydrogen peroxide		-699,597	-50,213
-Maintenance		-3,743	-3,743
-Electricity		-12,136	-12,136
-Total Oper Costs		-811,941	-162,556
-Capital Costs		-74,866	
Cash Flow		-886,807	-162,556

Sensitivity Analysis

Case Number	Capital Costs	Net Present Value	ROR
1	25.00	-993,213	0.00
2	50.00	-1,011,929	0.00
3	75.00	-1,030,646	0.00
4	100.00	-1,049,362	0.00
5	125.00	-1,068,079	0.00
6	150.00	-1,086,795	0.00
7	175.00	-1,105,512	0.00
8	200.00	-1,124,228	0.00

Case Number	Electricit	Net Present Value	ROR
1	25.00	-1,031,158	0.00
2	50.00	-1,037,226	0.00
3	75.00	-1,043,294	0.00
4	100.00	-1,049,362	0.00
5	125.00	-1,055,430	0.00
6	150.00	-1,061,498	0.00
7	175.00	-1,067,566	0.00
8	200.00	-1,073,634	0.00

Case Number	H2O2 Cost	Net Present Value	ROR
1	25.00	-487,005	0.00
2	50.00	-674,458	0.00
3	75.00	-861,910	0.00
4	100.00	-1,049,362	0.00
5	125.00	-1,236,815	0.00
6	150.00	-1,424,267	0.00
7	175.00	-1,611,720	0.00
8	200.00	-1,799,172	0.00

Case Number	Labor	Net Present Value	ROR
1	25.00	-904,666	0.00
2	50.00	-952,898	0.00
3	75.00	-1,001,130	0.00
4	100.00	-1,049,362	0.00
5	125.00	-1,097,594	0.00
6	150.00	-1,145,826	0.00
7	175.00	-1,194,058	0.00
8	200.00	-1,242,290	0.00

Case Number	Maintenanc	Net Present Value	ROR
1	25.00	-1,043,748	0.00
2	50.00	-1,045,619	0.00
3	75.00	-1,047,491	0.00
4	100.00	-1,049,362	0.00
5	125.00	-1,051,234	0.00
6	150.00	-1,053,106	0.00
7	175.00	-1,054,977	0.00
8	200.00	-1,056,849	0.00

Sensitivity Analysis

Case Number	Chem/Cyan Ratio	Net Present Value	ROR
1	25.00	-487,005	0.00
2	50.00	-674,458	0.00
3	75.00	-861,910	0.00
4	100.00	-1,049,362	0.00
5	125.00	-1,236,815	0.00
6	150.00	-1,424,267	0.00
7	175.00	-1,611,720	0.00
8	200.00	-1,799,172	0.00

In situ Chlorination Method

Cash Flows

PWC =1,151,388.86

Year	Time 0	1	2
-Alkaline		-247,652	-27,557
-Hypochlorite		-518,889	-57,738
-Maintenance		-3,743	-3,743
-Electricity		-12,136	-12,136
-Labor		-96,464	-96,464
-Total Oper Costs		-878,885	-197,638
-Capital Costs		-74,866	
Cash Flow		-953,751	-197,638

Sensitivity Analysis

Case Number	Capital Costs	Net Present Value	ROR
1	25.00	-1,095,239	0.00
2	50.00	-1,113,956	0.00
3	75.00	-1,132,672	0.00
4	100.00	-1,151,389	0.00
5	125.00	-1,170,105	0.00
6	150.00	-1,188,822	0.00
7	175.00	-1,207,538	0.00
8	200.00	-1,226,255	0.00

Case Number	Electricit	Net Present Value	ROR
1	25.00	-1,133,185	0.00
2	50.00	-1,139,253	0.00
3	75.00	-1,145,321	0.00
4	100.00	-1,151,389	0.00
5	125.00	-1,157,457	0.00
6	150.00	-1,163,525	0.00
7	175.00	-1,169,593	0.00
8	200.00	-1,175,661	0.00

Case Number	Hypochlori Cost	Net Present Value	ROR
1	25.00	-718,918	0.00
2	50.00	-863,075	0.00
3	75.00	-1,007,232	0.00
4	100.00	-1,151,389	0.00
5	125.00	-1,295,546	0.00
6	150.00	-1,439,703	0.00
7	175.00	-1,583,860	0.00
8	200.00	-1,728,016	0.00

Case Number	Labor	Net Present Value	ROR
1	25.00	-1,006,693	0.00
2	50.00	-1,054,925	0.00
3	75.00	-1,103,157	0.00
4	100.00	-1,151,389	0.00
5	125.00	-1,199,621	0.00
6	150.00	-1,247,853	0.00
7	175.00	-1,296,085	0.00
8	200.00	-1,344,317	0.00

Case Number	Maintenanc	Net Present Value	ROR
1	25.00	-1,145,774	0.00
2	50.00	-1,147,646	0.00
3	75.00	-1,149,517	0.00
4	100.00	-1,151,389	0.00
5	125.00	-1,153,261	0.00
6	150.00	-1,155,132	0.00
7	175.00	-1,157,004	0.00
8	200.00	-1,158,875	0.00

Case Number	Chem/Cyan Ratio	Net Present Value	ROR
1	25.00	-512,512	0.00
2	50.00	-725,471	0.00
3	75.00	-938,430	0.00
4	100.00	-1,151,389	0.00
5	125.00	-1,364,348	0.00
6	150.00	-1,577,307	0.00
7	175.00	-1,790,266	0.00
8	200.00	-2,003,225	0.00

Case Number	Sodium hydrox. Cost	Net Present Value	ROR
1	25.00	-944,982	0.00
2	50.00	-1,013,785	0.00
3	75.00	-1,082,587	0.00
4	100.00	-1,151,389	0.00
5	125.00	-1,220,191	0.00
6	150.00	-1,288,993	0.00
7	175.00	-1,357,795	0.00
8	200.00	-1,426,597	0.00

Ex situ Biological Method

Cash Flows

PWC = $919,312.94

Year	Time 0	1	2	3
-Labor		-123,464	-123,464	-123,464
-Phosphate		-14,827	-14,827	-7,414
-Maintenance		-20,366	-20,366	-20,366
-Electricity		-12,136	-12,136	-6,068
-Growth Medium		-13,100		
-Total Oper Costs		-183,893	-170,793	-157,311
-Capital Costs		-407,316		
Cash Flow		-591,209	-170,793	-157,311

Sensitivity Analysis

Case Number	Capital Costs	Net Present Value	ROR
1	25.00	-613,826	0.00
2	50.00	-715,655	0.00
3	75.00	-817,484	0.00
4	100.00	-919,313	0.00
5	125.00	-1,021,142	0.00
6	150.00	-1,122,971	0.00
7	175.00	-1,224,800	0.00
8	200.00	-1,326,629	0.00

Case Number	Electricit	Net Present Value	ROR
1	25.00	-896,558	0.00
2	50.00	-904,143	0.00
3	75.00	-911,728	0.00
4	100.00	-919,313	0.00
5	125.00	-926,898	0.00
6	150.00	-934,483	0.00
7	175.00	-942,068	0.00
8	200.00	-949,653	0.00

Case Number	Growth Medium Cost	Net Present Value	ROR
1	25.00	-909,488	0.00
2	50.00	-912,763	0.00
3	75.00	-916,038	0.00
4	100.00	-919,313	0.00
5	125.00	-922,588	0.00
6	150.00	-925,863	0.00
7	175.00	-929,138	0.00
8	200.00	-932,413	0.00

Case Number	Inoculum Volume	Net Present Value	ROR
1	25.00	-909,488	0.00
2	50.00	-912,763	0.00
3	75.00	-916,038	0.00
4	100.00	-919,313	0.00
5	125.00	-922,588	0.00
6	150.00	-925,863	0.00
7	175.00	-929,138	0.00
8	200.00	-932,413	0.00

Case Number	Labor	Net Present Value	ROR
1	25.00	-641,519	0.00
2	50.00	-734,117	0.00
3	75.00	-826,715	0.00
4	100.00	-919,313	0.00
5	125.00	-1,011,911	0.00
6	150.00	-1,104,509	0.00
7	175.00	-1,197,107	0.00
8	200.00	-1,289,705	0.00

Case Number	Maintenanc	Net Present Value	ROR
1	25.00	-873,490	0.00
2	50.00	-888,764	0.00
3	75.00	-904,039	0.00
4	100.00	-919,313	0.00
5	125.00	-934,587	0.00
6	150.00	-949,862	0.00
7	175.00	-965,136	0.00
8	200.00	-980,410	0.00

Case Number	Phosphate Cost	Net Present Value	ROR
1	25.00	-891,512	0.00
2	50.00	-900,779	0.00
3	75.00	-910,046	0.00
4	100.00	-919,313	0.00
5	125.00	-928,580	0.00
6	150.00	-937,847	0.00
7	175.00	-947,114	0.00
8	200.00	-956,380	0.00

Case Number	Phosphate Conc.	Net Present Value	ROR
1	25.00	-891,512	0.00
2	50.00	-900,779	0.00
3	75.00	-910,046	0.00
4	100.00	-919,313	0.00
5	125.00	-928,580	0.00
6	150.00	-937,847	0.00
7	175.00	-947,114	0.00
8	200.00	-956,380	0.00

INCO Air-SO$_2$ Method

Cash Flows

PWC =1,294,464.89

Year	Time 0	1	2	3
-INCO Royalty		-109,810	-17,659	-24
-Sodium metabisulfite		-301,676	-48,513	-65
-Sodium hydroxide		-351,955	-56,598	-76
-Electricity		-12,500	-12,500	-6,250
-Maintenance		-3,802	-3,802	-3,802
-Labor		-96,464	-96,464	-96,464
-Total Oper Costs		-876,206	-235,536	-106,681
-Capital Costs		-76,042		
Cash Flow		-952,248	-235,536	-106,681

Sensitivity Analysis

Case Number	Capital Costs	Net Present Value	ROR
1	25.00	-1,237,433	0.00
2	50.00	-1,256,444	0.00
3	75.00	-1,275,454	0.00
4	100.00	-1,294,465	0.00
5	125.00	-1,313,475	0.00
6	150.00	-1,332,486	0.00
7	175.00	-1,351,496	0.00
8	200.00	-1,370,507	0.00

Case Number	INCO Royalty	Net Present Value	ROR
1	25.00	-1,198,846	0.00
2	50.00	-1,230,719	0.00
3	75.00	-1,262,592	0.00
4	100.00	-1,294,465	0.00
5	125.00	-1,326,338	0.00
6	150.00	-1,358,211	0.00
7	175.00	-1,390,084	0.00
8	200.00	-1,421,957	0.00

Case Number	Electricit	Net Present Value	ROR
1	25.00	-1,271,027	0.00
2	50.00	-1,278,840	0.00
3	75.00	-1,286,652	0.00
4	100.00	-1,294,465	0.00
5	125.00	-1,302,277	0.00
6	150.00	-1,310,090	0.00
7	175.00	-1,317,902	0.00
8	200.00	-1,325,715	0.00

Case Number	Labor	Net Present Value	ROR
1	25.00	-1,077,421	0.00
2	50.00	-1,149,769	0.00
3	75.00	-1,222,117	0.00
4	100.00	-1,294,465	0.00
5	125.00	-1,366,813	0.00
6	150.00	-1,439,161	0.00
7	175.00	-1,511,509	0.00
8	200.00	-1,583,857	0.00

Case Number	Chem/ Cyanide Ratio	Net Present Value	ROR
1	25.00	-629,684	0.00
2	50.00	-851,278	0.00
3	75.00	-1,072,871	0.00
4	100.00	-1,294,465	0.00
5	125.00	-1,516,059	0.00
6	150.00	-1,737,652	0.00
7	175.00	-1,959,246	0.00
8	200.00	-2,180,839	0.00

Case Number	Sodium hydroxide	Net Present Value	ROR
1	25.00	-987,993	0.00
2	50.00	-1,090,150	0.00
3	75.00	-1,192,308	0.00
4	100.00	-1,294,465	0.00
5	125.00	-1,396,622	0.00
6	150.00	-1,498,779	0.00
7	175.00	-1,600,937	0.00
8	200.00	-1,703,094	0.00

Case Number	Sodium metabisulf	Net Present Value	ROR
1	25.00	-1,031,775	0.00
2	50.00	-1,119,338	0.00
3	75.00	-1,206,902	0.00
4	100.00	-1,294,465	0.00
5	125.00	-1,382,028	0.00
6	150.00	-1,469,592	0.00
7	175.00	-1,557,155	0.00
8	200.00	-1,644,718	0.00

Case Number	Maintenanc	Net Present Value	ROR
1	25.00	-1,285,910	0.00
2	50.00	-1,288,762	0.00
3	75.00	-1,291,613	0.00
4	100.00	-1,294,465	0.00
5	125.00	-1,297,316	0.00
6	150.00	-1,300,168	0.00
7	175.00	-1,303,020	0.00
8	200.00	-1,305,871	0.00

Ex situ Peroxide Method

Cash Flows

PWC =1,325,243.76

Year	Time 0	1	2	3
-Labor		-96,464	-96,464	-96,464
-Hydrogen peroxide		-791,859	-127,346	-171
-Maintenance		-3,743	-3,743	-3,743
-Electricity		-12,152	-12,152	-6,076
-Total Oper Costs		-904,218	-239,706	-106,454
-Capital Costs		-74,866		
Cash Flow		-979,084	-239,706	-106,454

Sensitivity Analysis

Case Number	Capital Costs	Net Present Value	ROR
1	25.00	-1,269,094	0.00
2	50.00	-1,287,811	0.00
3	75.00	-1,306,527	0.00
4	100.00	-1,325,244	0.00
5	125.00	-1,343,960	0.00
6	150.00	-1,362,677	0.00
7	175.00	-1,381,393	0.00
8	200.00	-1,400,110	0.00

Case Number	Electricit	Net Present Value	ROR
1	25.00	-1,302,459	0.00
2	50.00	-1,310,054	0.00
3	75.00	-1,317,649	0.00
4	100.00	-1,325,244	0.00
5	125.00	-1,332,839	0.00
6	150.00	-1,340,434	0.00
7	175.00	-1,348,029	0.00
8	200.00	-1,355,624	0.00

Case Number	Labor	Net Present Value	ROR
1	25.00	-1,108,200	0.00
2	50.00	-1,180,548	0.00
3	75.00	-1,252,896	0.00
4	100.00	-1,325,244	0.00
5	125.00	-1,397,592	0.00
6	150.00	-1,469,940	0.00
7	175.00	-1,542,288	0.00
8	200.00	-1,614,636	0.00

Case Number	Maintenanc	Net Present Value	ROR
1	25.00	-1,316,821	0.00
2	50.00	-1,319,629	0.00
3	75.00	-1,322,436	0.00
4	100.00	-1,325,244	0.00
5	125.00	-1,328,051	0.00
6	150.00	-1,330,859	0.00
7	175.00	-1,333,666	0.00
8	200.00	-1,336,474	0.00

Case Number	H2O2 Cost	Net Present Value	ROR
1	25.00	-635,712	0.00
2	50.00	-865,556	0.00
3	75.00	-1,095,400	0.00
4	100.00	-1,325,244	0.00
5	125.00	-1,555,088	0.00
6	150.00	-1,784,932	0.00
7	175.00	-2,014,776	0.00
8	200.00	-2,244,620	0.00

Case Number	Chem/Cyan Ratio	Net Present Value	ROR
1	25.00	-635,712	0.00
2	50.00	-865,556	0.00
3	75.00	-1,095,400	0.00
4	100.00	-1,325,244	0.00
5	125.00	-1,555,088	0.00
6	150.00	-1,784,932	0.00
7	175.00	-2,014,776	0.00
8	200.00	-2,244,620	0.00

Ex situ Chlorination Method

Cash Flows

PWC =1,354,433.13

Year	Time 0	1	2	3
-Labor		-96,464	-96,464	-96,464
-Hypochlorite		-553,028	-88,951	-127
-Maintenance		-3,743	-3,743	-3,743
-Electricity		-12,152	-12,152	-6,076
-Alkaline		-263,945	-42,454	-60
-Total Oper Costs		-929,332	-243,765	-106,470
-Capital Costs		-74,866		
Cash Flow		-1,004,198	-243,765	-106,470

Sensitivity Analysis

Case Number	Hypochlori Cost	Net Present Value	ROR
1	25.00	-872,854	0.00
2	50.00	-1,033,380	0.00
3	75.00	-1,193,907	0.00
4	100.00	-1,354,433	0.00
5	125.00	-1,514,960	0.00
6	150.00	-1,675,486	0.00
7	175.00	-1,836,012	0.00
8	200.00	-1,996,539	0.00

Sensitivity Analysis

Case Number	Chem/Cyan Ratio	Net Present Value	ROR
1	25.00	-643,009	0.00
2	50.00	-880,151	0.00
3	75.00	-1,117,292	0.00
4	100.00	-1,354,433	0.00
5	125.00	-1,591,574	0.00
6	150.00	-1,828,716	0.00
7	175.00	-2,065,857	0.00
8	200.00	-2,302,998	0.00

Case Number	Electricit	Net Present Value	ROR
1	25.00	-1,331,648	0.00
2	50.00	-1,339,243	0.00
3	75.00	-1,346,838	0.00
4	100.00	-1,354,433	0.00
5	125.00	-1,362,028	0.00
6	150.00	-1,369,623	0.00
7	175.00	-1,377,218	0.00
8	200.00	-1,384,813	0.00

Case Number	Labor	Net Present Value	ROR
1	25.00	-1,137,389	0.00
2	50.00	-1,209,737	0.00
3	75.00	-1,282,085	0.00
4	100.00	-1,354,433	0.00
5	125.00	-1,426,781	0.00
6	150.00	-1,499,129	0.00
7	175.00	-1,571,477	0.00
8	200.00	-1,643,825	0.00

Case Number	Maintenanc	Net Present Value	ROR
1	25.00	-1,346,011	0.00
2	50.00	-1,348,818	0.00
3	75.00	-1,351,626	0.00
4	100.00	-1,354,433	0.00
5	125.00	-1,357,241	0.00
6	150.00	-1,360,048	0.00
7	175.00	-1,362,856	0.00
8	200.00	-1,365,663	0.00

Case Number	Sodium hydrox. Cost	Net Present Value	ROR
1	25.00	-1,124,588	0.00
2	50.00	-1,201,203	0.00
3	75.00	-1,277,818	0.00
4	100.00	-1,354,433	0.00
5	125.00	-1,431,048	0.00
6	150.00	-1,507,663	0.00
7	175.00	-1,584,278	0.00
8	200.00	-1,660,893	0.00

Case Number	Capital Costs	Net Present Value	ROR
1	25.00	-1,298,284	0.00
2	50.00	-1,317,000	0.00
3	75.00	-1,335,717	0.00
4	100.00	-1,354,433	0.00
5	125.00	-1,373,150	0.00
6	150.00	-1,391,866	0.00
7	175.00	-1,410,583	0.00
8	200.00	-1,429,299	0.00

APPENDIX H: PGY RECIPE

For 1 liter of full-strength PGY broth:

5 g Bacto-peptone
0.5 g yeast extract
3.2 g glycerol
1 liter tap water